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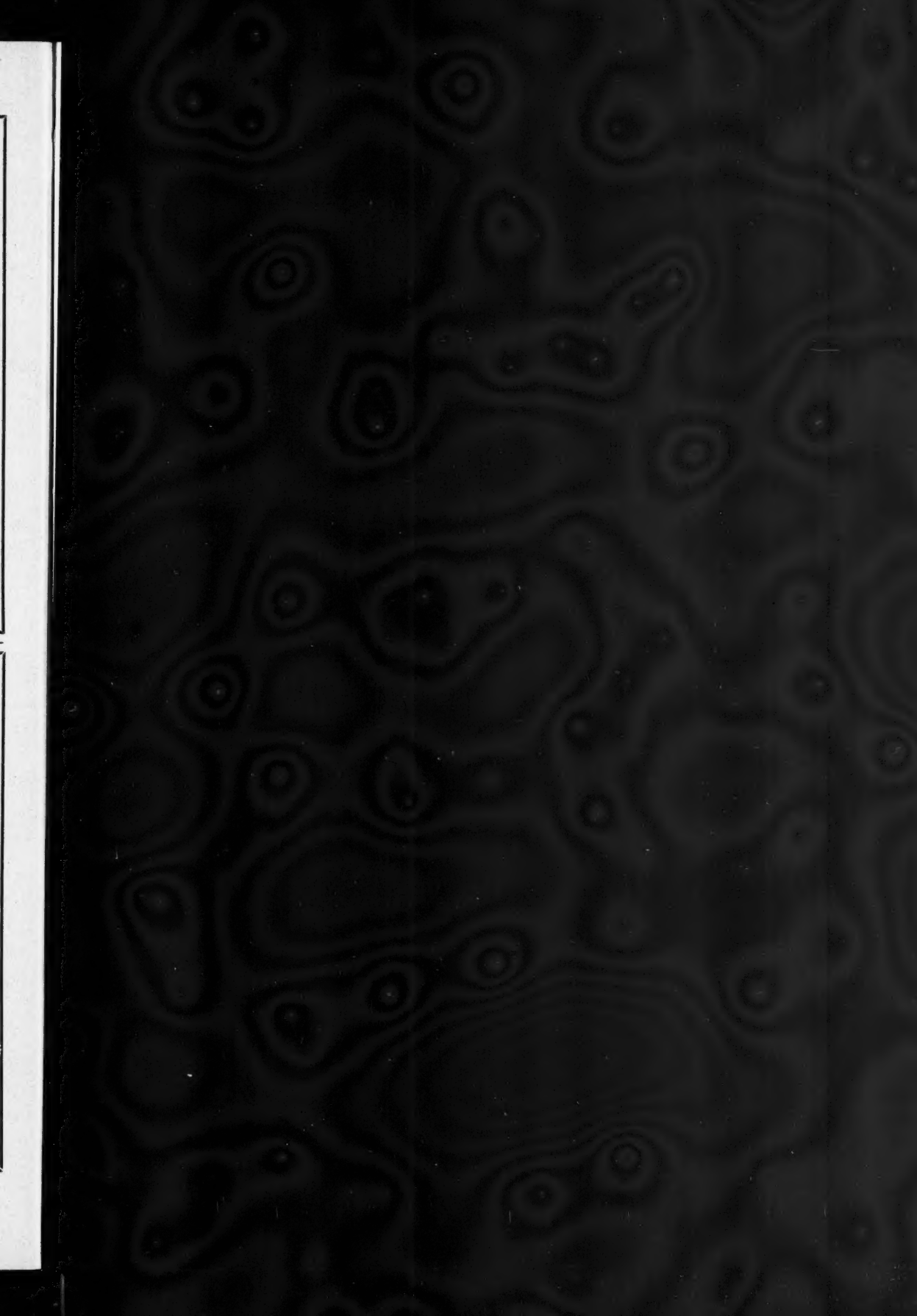

DIFFERENCES EFFECTED IN THE PROTEIN CONTENT OF GRAIN BY APPLICATIONS OF NITROGEN MADE AT DIFFERENT GROWING PERIODS OF THE PLANTS

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In a previous paper (1), the writer has shown how the protein content of wheat (White Australian) is markedly influenced by the supply of nitrogen available to the plants at certain phases of their growth. That investigation led to further experimentation with other cereals to see to what extent the protein content of other grains might be affected. The investigation has now proceeded so far that some of the results can be given publication.

Briefly described the cultural methods employed to obtain high protein grain were as follows:

One-gallon stone jars were filled with a soil low in nitrogen which is known locally as Oakley sand. This soil as taken from the field had a low crop producing power for cereals, but it responded readily in large crop production when treated with a moderate application of $NaNO_3$ or $(NH_4)_2SO_4$. The jars filled with soil, were seeded to the following cereals:

Spring wheat (White Australian)
Oats (Texas Red)
Winter wheat (Turkey Red)
Rye (variety not known but considered a pure strain)

At the time of planting, one set of three jars of each of the different cereals received per jar, in the form of $NaNO_3$, an application of 250 mgm. of nitrogen (equivalent to 100 lbs. per acre). About two weeks later, when the plants were between 2 and 3 inches high, another set of three cultures of the four cereals named was similarly treated. At later periods other sets of three cultures of each of the four cereals received similar applications of $NaNO_3$. The complete treatment is outlined in table 1.

The essential difference in the treatments of the cultures was that of the time in the growth period of the plants when nitrogen was applied. That this was a very important factor in the physiological processes concerned in the growth of some of the cereals tested was shown by the results obtained. Differences in tillering of the plants, differences in the length of the growing period of the plants, differences in chemical composition of the grain, and differences in total dry matter production resulted from the treatments.

The plants were harvested when fully mature, and the grain was threshed, weighed and graded. Nitrogen determinations were made of samples from all cultures. This was then computed to give the protein content of the grain.

The data given in this paper deals only with the differences in the protein content of grain obtained from the sets treated with $NaNO_3$. Ammonium

sulfate also was used as the source of nitrogen for other sets, but because the results obtained from these are in essence similar to the results obtained from the $NaNO_3$ treated sets, they need not be given here. The tables have been prepared to show both the days after planting and days before harvest when $NaNO_3$ was applied to the cultures. The protein content of the grain from each culture is given to show the extent of the variations obtained, and the weight of 100 kernels from each culture (excepting those of oats) gives an indication of the extent the grain was filled.

Table 2 gives the results obtained with a spring wheat (White Australian). The seed wheat used for this investigation was low in protein and high in starch,—a characteristic soft wheat. The table shows that the cultures which received the latest application of $NaNO_3$ produced wheat that had the highest protein content of all of the set. This wheat was of amber color, flinty and hard. The cultures that received $NaNO_3$ at the time of planting and 17 days thereafter, produced wheat of whitish yellow color, low in protein, high in starch—a typical soft wheat. The data show a progressive increase in the

TABLE 1

Data showing age of spring wheat cultures when nitrogen was applied

SET NUMBER	DATE OF PLANTING	DATE OF TREATMENT	AGE OF PLANTS WHEN $NaNO_3$ WAS APPLIED	$NaNO_3$ ADDED PER CULTURE
			days	*mgm.*
1	Nov. 14, 1919	Nov. 14, 1919		250
2	Nov. 14, 1919	Dec. 1, 1919	17	250
3	Nov. 14, 1919	Dec. 16, 1919	33	250
4	Nov. 14, 1919	Jan. 1, 1920	48	250
5	Nov. 14, 1919	Jan. 24, 1920	72	250
6	Nov. 14, 1919	March 2, 1920	110	250

protein content of the wheat, that corresponds to each increase in the length of time after planting when nitrogen was applied, or if otherwise stated, as corresponding with each decrease in the length of the period before harvest when $NaNO_3$ was applied. This seems to be evidence that the two circumstances are related as cause and effect. It shows furthermore that a very important condition for the production of high protein wheat as obtained in this investigation is one that requires sufficient supply of available nitrogen at what appears to be certain important, perhaps, critical growth periods of the plants. This supply seemingly must be in excess of the minimum requirement needed for the formation and filling of the kernels. Table 2 shows that the protein content of the spring wheat (White Australian) is subject to a wide variation. This variation, as the investigation shows, is largely accounted for in the differences of conditions in the external environment of the plants at certain phases of their growth. The extreme differences in percentage of protein of the grain, from plants that received nitrogen at the time of planting on the one hand, and those that received nitrogen 110 days after

planting on the other, and the good correlation obtained between the differences in the protein content of the grain and the corresponding treatments, is evidence that soft wheat and hard wheat, of this particular variety, is largely due to factors operative in the nutrition of the plants.

Table 3 gives the results obtained by the treatments on the protein content of winter wheat. The seed used for the tests was relatively high in protein, the grain being classed as hard wheat. The results given in this table are

TABLE 2

Effect of $NaNO_3$ on the protein content of spring wheat (White Australin) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	201	4.57	8.9	
2		201	4.27	8.6	8.9
3		199	4.86	9.1	
4	17	183	5.11	9.6	
5	17	178	5.22	9.3	9.2
6	17	183	4.90	8.8	
7	33	162	5.11	11.3	
8	33	162	5.00	10.1	10.6
9	33	162	5.18	10.4	
10	48	147	5.22	10.7	
11	48	152	5.15	11.7	11.4
12	48	152	4.95	11.7	
13	72	135	4.92	13.1	
14	72	135	4.75	13.2	13.0
15	72	135	4.68	12.8	
16	110	121	4.00	14.7	
17	110	121	4.43	15.3	15.2
18	110	121	4.10	15.6	

* Date of planting was November 14, 1919.

decidedly different from those obtained with spring wheat in that the data do not show (with the exception of that of the last treatment), that the time of application of $NaNO_3$ had any clearly definite effects upon the per cent of protein this wheat contained. That this winter wheat did not respond to the treatment as did the spring wheat, seemingly must be due to differences in the physiology of the two kinds of wheat. Winter wheat is characterized by a period of relative dormancy of growth before the plants stool or produce culms, but spring wheat is not. Under the conditions of this investigation,

spring wheat usually stools two or three weeks after the plants are up. It seems probable that the differences in the results obtained from the two classes of wheat can be partly accounted for by the relative dormancy of growth of the winter wheat. Due to this relative dormancy of growth of winter wheat the intervals of time at which $NaNO_3$ was applied to the cultures did not represent equal or comparable differences in the growth phases of winter wheat as similar intervals of time between applications of $NaNO_3$ represented dif-

TABLE 3

Effect of $NaNO_3$ on the protein content of winter wheat (variety, Turkey Red) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	226	3.55	14.3	
2		226	3.72	15.0	14.6
3		232	3.86	14.6	
4	21	205	3.66	14.7	
5	21	205	3.97	13.0	13.8
6	21	205	4.10	13.6	
7	36	190	3.74	14.7	
8	36	179	4.09	14.0	14.7
9	36	180	4.13	15.4	
10	60	155	3.56	13.7	
11	60	166	3.13	13.7	13.4
12	60	166	3.88	12.8	
13	81	145	3.43	13.3	
14	81	152	3.51	14.5	14.3
15	81	152	3.18	15.0	
16	109	124	3.50	17.5	
17	109	124	3.00	18.6	17.9
18	109	124	3.21	17.5	

* Date of planting was November 18, 1919.

ferent growth phases in spring wheat. Table 3 shows that only in case of the last treatment, that is when the application of $NaNO_3$ was made 109 days after planting, the grain produced was appreciably higher in protein than that produced from any of the other cultures of winter wheat. The grain obtained from the cultures that received the last application of $NaNO_3$ was approximately 25 per cent higher in protein than was that obtained from any other set of this wheat.

Table 4 gives the results obtained on the protein content of oats and shows that a progressive increase resulted from the treatments. In case of the last application of nitrogen made to a set of cultures 108 days after planting, oats were produced that averaged 17.2 per cent protein, which was an increase of approximately 130 per cent over that obtained from the cultures that received nitrogen at the time of planting. The data show that the oats and the spring

TABLE 4

Effect of $NaNO_3$ on the protein content of oats (variety Texas Red) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*		*per cent*	*per cent*
1	At time of planting	197	Not determined	7.5	
2(Lost)		...		....	7.5
3		197		7.5	
4	19	178		7.7	
5	19	178		7.9	8.0
6	19	178		8.5	
7	33	164		7.9	
8	33	164		8.5	8.5
9	33	164		9.0	
10	48	157		9.3	
11	48	157		9.4	9.6
12	48	157		10.0	
13	69	136		9.8	
14	69	136		11.3	10.8
15	69	136		11.2	
16	90	121		12.8	
17	90	121		13.0	12.7
18	90	121		12.3	
19	108	111		18.2	
20	108	111		15.8	17.2
21	108	111		17.5	

* Date of planting was November 6, 1919.

wheat tested exhibited certain common properties; one being that the physiological status of the plant as indicated by its growth phases is a very important factor, which affects the power and capacity of the plant to absorb and to utilize nitrogen most efficiently for the production of high protein grain.

Table 5 gives the results obtained in the experiments with rye. The protein content of the grain of the first four sets of cultures that received $NaNO_3$

was not affected by the treatment. The grain obtained from the three last sets of cultures that received $NaNO_3$ gave a progressive increase in protein with each treatment so that the cultures which received nitrogen 133 days after planting, or 108 days before harvest, produced grain that averaged 14.0 per cent protein. This is an increase of approximately 50 per cent over that of the grain obtained from cultures that received $NaNO_3$ at the time of planting. So far as the magnitude of difference in the protein content of rye as

TABLE 5

Effect of $NaNO_3$ on the protein content of rye (variety unknown) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	199	2.90	9.4	
2		204			9.5
3		204	3.04	9.6	
4	19	185	3.23	9.4	
5	19	185	3.16	8.7	9.0
6	19	183	3.08	8.8	
7	28	168	3.01	9.5	
8	28	168	2.81	10.5	9.7
9	28	171	2.42	9.1	
10	43	156	3.51	9.8	
11	43	156	2.80	9.6	9.8
12	43	156	2.93	10.1	
13	74	135	2.50	10.9	10.9
14	74	140	2.68	10.9	
15	105	130	3.60	11.9	12.0
16	105	130	3.27	12.1	
17	133	108	3.10	13.5	14.0
18	133	108	2.63	14.5	

* Date of planting was November 11, 1919.

affected by the treatment is concerned, rye occupies a place between winter wheat on one hand, and spring wheat and oats on the other. Presumably this is to be accounted for in physiology and growth habits of the plant, rye having a period of relative dormancy of growth like that of winter wheat but of shorter duration, and being unlike spring wheat and oats. The per cent of protein of the grain obtained from the first four sets of cultures was not affected by the addition of soluble nitrogen, presumably due to the fact that this

nutrient was added to the plants when they were in a period of relative dormancy of growth. A correlation in the increase of the protein content of the grain and the different treatments applied after the plants had passed through the period of relative dormancy of growth seems to be proof that rye plants have a growth phase when they absorb and utilize nitrogen most efficiently for the production of high protein grain.

Inspection of the four tables show that the size of the grains as indicated by the weight per 100 kernels was not markedly affected by the treatments. Applying $NaNO_3$ early or relatively late in the growing period of the plants, did not apparently affect the extent to which the kernels were filled. The yield per culture was, as already stated, markedly influenced by the treatment. This phase of the investigation, however, will be treated in another paper.

The results of this investigation show that the chemical composition of grain such as the protein content of some of the important cereals can be markedly affected by factors involved in the nutrition of the plants. Furthermore the data show that variations in the protein content of grain are not always due to unknown genetic factors, but they may be definite non-inheritable responses of the plant to certain conditions of its external environment. It seems, from a critical analysis of the data, that in some of the nutritional processes of the plants studied are to be found certain conditions that might account for properties or characters which heretofore have been considered to be of genetic origin.

REFERENCE

(1) GERICKE, W. F. 1920 On the protein content of wheat. *In* Science, v. 52, p. 446–447.

SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 1

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INTRODUCTORY

For at least three-quarters of a century the effect of compounds of arsenic upon plant growth has been a subject of scientific investigation. It was early recognized that arsenic is quite generally found in animal tissue; and, since the animal must have obtained the arsenic from its food, it was soon found that arsenic is present in minute amounts in many plants. Later investigations (2) have shown arsenic to be very widely distributed in the soil, and to have been derived in this case from the original rocks from which the soil was formed.

The fact that arsenic was found to be so generally present in animal and vegetable tissues raised the question as to whether or not arsenic is essential to the life processes. Gautier and Clausmann (1) failed to find arsenic in *some* plants and concluded from this fact that arsenic is not essential to vegetable life. But the more intimately life processes are investigated the more apparent it is becoming that very minute quantities of substances may have a very decided effect on those processes. For example, note the decided effect which the minute amount of iodine in the thyroid gland has on animal life processes. The more recent investigations are thus making it appear that it is still too early to draw definite and final conclusions as to the rôle of arsenic in plant and animal life; and more refined methods of investigation may very probably be expected to reveal the more general presence of arsenic in plants than heretofore found.

But even though arsenic in minute quantities may or may not be useful to plant or animal life, it has been abundantly proved that it is a strong poison to both plants and animals if the quantity absorbed is large. Some years ago it was reported (5 and 6) that orchard trees were dying in Colorado as a result of arsenic poisoning. Since the practice of spraying is resulting in the application of considerable quantities of arsenical compounds to the soil and since smelters in some localities belch forth large amounts of arsenic oxides annually, it thus becomes important from a practical point of view to have more definite information as to the effects of arsenic on plants. Dr. Greaves (2, 3, 4) worked on this problem and has published some of his results in several papers. His work at first dealt with some chemical phases, but later it has dealt with the effects of arsenical compounds on the microörganisms of the soil, ammonifiers and nitrifying organisms.

In 1912 the chemical phase of this investigation was turned over to the author of this paper, who devised a plan of attacking the problem.

It was proposed to determine the solubility of lead arsenate and other compounds of arsenic, likely to be used for spraying purposes, in solutions of the inorganic salts commonly occurring in the soil, a determination being made with each pure salt. Two series of concentrations were to be used, one representing the concentrations which might be expected in an ordinarily rich soil and the other representing concentrations such as might be expected in strongly "alkali" soils. It was also proposed to determine these solubilities in mixtures of all the individual salts used in each of the two series mentioned. Some humus was to be prepared and, after removal of the ammonia, it was to be used in some more determinations with the two series of solutions to test the effect of organic matter of the soil in conjunction with the soluble salts on the solubility of the arsenic compounds. Soils were also to be collected from the principal fruit growing sections of the State. A definite quantity of each soil was to be treated with a definite volume of water and the solubility of the arsenic compounds in these mixtures was to be determined. It was also proposed to build concrete, water tight vessels, sufficiently large for the roots of a mature tree. These were to be built so the top was flush with the surface of the ground, and filled with soil the composition of which was known. Some of the orchard trees, such as apple, peach, pear, etc., were to be grown to maturity in these tanks and then subjected to the usual spraying treatment given to orchards. This treatment was to be continued for about five years or more after the tree commenced to bear, and the effects of the spraying treatment on the tree, the fruit, and the soil were to be noted. A series of tests was also to be made upon a number of young trees and shrubs grown in small vessels. For instance, about twenty to forty apple trees were to be grown in this way. These were to be divided into ten sets of two or four trees each. Each set, except one tree for control, was then to be treated with solutions containing a definite amount of sodium arsenate, each set receiving a different amount. The effect of the treatment was to be studied by means of the appropriate observations and analyses. All kinds of ordinary orchard trees were to be treated in this way. The experiment as planned was intended to be a somewhat exhaustive study of the effects and nature of arsenical poisoning on plants. Results of value from both a practical and scientific standpoint were expected. Some of the work planned has been accomplished, and this paper sets forth these results.

The methods of analysis in such work must be delicate, reliable, and as rapid and simple as possible. To select or develop the most suitable analytical methods was in itself no small task. So before commencing on the experimental part of the investigation the author made a comparative study of the methods for determining arsenic. This resulted in a decision to use the Marsh method as modified by Greaves (3) of this station and the Williamson method mentioned by Sutton (8) as modified by the author of this paper. The Williamson method is fraught with some difficulties, as a reference to the recent literature will show. The means of satisfactorily overcoming these difficulties were worked out in this laboratory. Thoroughly understood, the method becomes a rapid and excellent one, but not suitable for the determination of such minute quantities of arsenic as is the Marsh method. For further information about the analytical methods used in this work, reference must be made to the earlier paper of Greaves (3) and to the author[1] for unpublished information concerning the modified Williamson method.

[1] A paper by the author describing his modification of the Williamson method was accepted for publication by the *Zeitschrift für Analytische Chemie* in the early summer of 1914, but the outbreak of the war seems to have prevented its publication.

EXPERIMENTAL

Solubility of lead arsenate in salt solutions

The modified Williamson method was used for determining the arsenic dissolved in the salt solutions and the soil solutions. The modified Marsh method was used for determining the arsenic absorbed by the plants in the pot experiment, which will be reported in another paper. The lead arsenate used for the solubility determinations was *Sherwin Williams*, guaranteed to contain not less than 25 per cent of arsenic, and not more than 1½ per cent of water soluble arsenic. It was an impalpable dry powder.

As stated above, solubility in solutions of each salt of two concentrations was determined. Potassium carbonate was used as a starting point for determining the concentrations. The weaker potassium carbonate solution contained 0.10 per cent of K_2CO_3, the stronger one contained 0.50 per cent of K_2CO_3. The concentrations of the other salt solutions were then made such that the basic elements were present in all the solutions in chemically equivalent amounts. For example, the weaker series of solutions contained in 2 liters, 2.0000 gm. of K_2CO_3, 2.1580 gm. of KCl, 1.5340 gm. of Na_2CO_3 etc.; the stronger series of solutions contained 10.0000 gm. of K_2CO_3, 10.7900 gm. KCl., 7.6700 gm. Na_2CO_3 etc. The solutions of the common salts of potassium, sodium, ammonium, calcium and magnesium prepared in this way were then treated with an excess of lead arsenate and shaken two or more times per day for three weeks or more. The solutions were filtered, one liter treated with 10 cc. of HNO_3 and 10 cc. of H_2SO_4 and evaporated to white fumes. The residue was cooled, the sides of the vessel washed down with water and again evaporated to white fumes. This dilution and re-evaporation are necessary to break down the nitrosyl sulfuric acid and thus completely remove oxides of nitrogen. The dissolved arsenic was then determined.

Some difficulties were encountered in this work and as yet no satisfactory means of entirely overcoming them has been found. Solutions such as Na_2CO_3 which give an alkaline reaction on account of hydrolysis, persistently hold the lead arsenate in colloidal solution and suspension. No way of filtering out this colloidal material was found; after the best filtration possible such solutions were still opalescent. Filtration through Chamberlain-Pasteur filters was not feasible because these retained arsenic which was in true solution. The best way found was to filter through paper. By repeating the filtration several times through the same paper the pores became somewhat clogged with the lead arsenate, and a somewhat clearer filtrate resulted. It was found by Stewart (7) that a good way to filter humus was through paper covered with a thin layer of the soil from which the humus has been extracted. The two cases are identical in principle.

The length of time required to establish equilibrium between the dissolved and undissolved lead arsenate was also an obstacle to rapid work. But even shaking the solutions containing lead arsenate for six days in a shaking machine failed to establish equilibrium so that duplicates would agree satis-

factorily. Variations in temperature also result in slightly different values for the solubility.

The solubility data obtained for the various salt solutions are shown in table 1. The table is in three parts, A, B and C. Part A shows the solubility of the lead arsenate in the weaker concentrations mentioned above; part B, in the stronger concentrations; and part C, in tap water, distilled water and two mixtures of salts. Mixture 1 represents a mixture of the salts of the concentrations used in part A, and Mixture 2, a mixture of the salts of the concentrations used in part B. Some of the salts were precipitated out of solution

TABLE 1

Arsenic dissolved in the form of lead arsenate in various salt solutions

	CARBONATES	CHLORIDES	NITRATES	BICARBONATES	SULFATES	BISULFATES	PHOSPHATES	
							Secondary	Primary
A. Weaker concentrations								
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
K	165.2	39.3	4.5	139.9	4.6	241.9	174.2	29.1
Na	184.8	51.7	5.3	136.4	5.9	260.9	168.9	11.8
NH_4	175.6	37.0	3.4	136.4	3.5	271.5	177.6	18.7
Ca	73.4	54.7	2.0		5.6	296.3	73.9	36.6
Mg	4.1	36.6	3.5	37.4	4.5	268.5	93.5	21.0
B. Stronger concentrations								
K	115.9	156.0	8.1	99.8	5.8	647.2	292.9	78.0
Na	131.8	130.5	13.5	96.7	5.6	1016.9	357.0	84.6
NH_4	119.2		5.4	162.5		838.3	392.1	63.0
Ca	36.5	105.4	4.3	13.5	4.2	615.2	57.7	67.5
Mg	20.1	101.2	3.5	85.4	2.6	572.3	46.1	38.0

C. Other solvents	*p.p.m.*
Tap water	33.8
Distilled water	3.0
Mixture 1	183.8
Mixture 2	324.9

in both mixtures. The figures in the table represent milligrams of arsenic in a liter of solution.

In the case of sodium, potassium and ammonium carbonates and probably some of the alkali phosphates, the data represent some material that is in colloidal solution as well as that in true solution. In each case the figure in the table represents the average of two to ten determinations. It will be noted that the lead arsenate is only very slightly soluble in distilled water, 3 parts per million. In the presence of nitrates and sulfates the solubility does not appear to be very materially different from that in distilled water, there being possibly a very slight increase in solubility in the presence of these salts. Since lead sulfate is a very insoluble compound, the ionic theory would lead

one to expect such a result as appears in the table in the sulfate column; but as lead nitrate is a soluble compound the ionic theory would *a priori* lead one to expect a larger solubility than appears in the nitrate column in the table. Since a great number of analyses were made from which the data in the table are condensed, there can be no doubt that lead arsenate is not materially more soluble in nitrate solutions than in pure water. The data show that the neutral chlorides increase the solubility of the lead arsenate considerably. The difference between the effect of the chlorides and the nitrates is somewhat surprising, and must apparently be attributed to the specific nature of these salts modifying the solvent. That is, chloride solutions and nitrate solutions have different solvent powers in much the same way that water and alcohol have. As is to be expected, the acid salts such as the bicarbonates, the bisulfates and the acid phosphates cause considerably larger quantities of the lead arsenate to go into solution. Increasing the concentration of the chlorides, the bisulfates and the phosphates also increases the amount of arsenic dissolved; but in the case of the sulfates and nitrates increased concentration appears to have no effect, while in the case of the carbonates and bicarbonates increased concentration as used in these experiments causes a decrease in the amount of arsenic dissolved. Ten times as much lead arsenate was dissolved in tap water as in distilled water. This, of course, shows to some extent what is to be expected to occur in the underground water. The tap water contains 212 parts of solids per million, mostly in the form of bicarbonates of calcium and magnesium.

Solubility of lead arsenate in soil solution

Soils were collected for this experiment in 1913 from the principal fruit-growing sections of the state. The amount and composition of the water-soluble material in these soils were determined. The treatment with arsenic was as follows: Two hundred fifty grams of each of the soils were treated with one liter of water. An excess of lead arsenate was added to this mixture and the amount of arsenic dissolved determined in the same manner as indicated above for the salt solutions. The data expressed in milligrams of arsenic per liter are in table 2. The soils collected represented light sandy loams, gravelly loams, rich and somewhat heavy loams, and uncultivated alkali land.

Data in table 2 have been grouped roughly according to soil types. The College Farm soil stands alone. The group of nine soils following includes in the main somewhat gravelly bench lands which are more representative of the fruit-growing districts than the other soils mentioned. Next is a group of five light sandy soils without gravel. This is a lighter type of soil than the preceding group. The fourth group includes nine good loam soils from some of the best farming lands in the State. Some of them tend to heaviness, but all are easily tillable. The last two soils in the table represent uncultivated "alkali" land west of Salt Lake City and between Tooele and Grantsville.

The data show that lead arsenate is much more soluble in the soil solution than in pure water, and that it has about the same solubility in the soil water

TABLE 2

Solubility of lead arsenate in soil solution

SOIL NUMBER	LOCALITY	SOIL TYPE	ARSENIC PER LITER	COMPOSITION OF SOIL SOLUTION						
				Total solids	Cl	CO_3 (and HCO_3)	SO_4	Ca	Mg	K
			mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
78032	College Farm	Gravelly bench loam	44.5	0.238						
78033	College Farm		43.1	0.238						
77939	East of Midvale 1 mi.	Coarse sandy loam	20.4	0.140		0.034	0.008	0.071	0.021	0.013
77942	Tooele (W. side)	Bench loam	23.8	0.214		0.050	0.014	0.084	0.059	0.014
77945	Tooele (N. E. side)		11.1	0.142		0.040	0.008	0.060	0.027	0.012
77949	S. E. of Provo	Black, gravelly loam	22.0	0.270		0.040	0.046	0.060	0.027	0.017
77950	Provo Bench (Anderson Bros. orchard)	Light gravelly loam*	16.0	0.108		0.030	0.025	0.044	0.017	0.013
77954	Orchard S. W. of Ogden		10.5	0.092		0.024	0.017	0.050	0.019	0.006
77956	Pleasant View near Hot Springs	Gravelly bench land	25.4	0.186		0.060	0.022	0.056	0.026	0.012
77962	Near S. city limits of Brigham City		11.1	0.218	0.028	0.040	0.019	0.044	0.027	0.012
77963	Near Brigham City depot		12.1	0.140		0.041	0.014	0.040	0.011	0.007
Averages			17.0	0.168		0.041	0.019	0.056	0.026	0.012
77951	2 mi. So. 77950	Light sandy loam	8.1	0.102		0.034	0.016	0.033	0.020	0.007
77946	Clearfield		9.6	0.146		0.022	0.012	0.036	0.027	0.008
77955	Five points		16.2	0.124		0.035	0.023	0.052	0.024	0.007
77958	North of Willard	Light loam	9.4	0.114		0.016	0.017	0.034	0.023	0.010
77960	Dewey (W. of Depot)		12.6	0.142		0.031	0.031	0.056	0.023	0.010
Averages			11.2	0.126		0.027	0.020	0.042	0.023	0.008

77938	West Jordan	Heavy loam	26.2	0.230		0.055	0.017	0.075	0.032	0.022
77940	North of Murray near Hussler's mill		23.1							
77944	Grantsville		31.8	0.220		0.061	0.016	0.056	0.031	0.007
77947	Farmington	Heavy black loam	19.8	0.164	0.071	0.014	0.010	0.056	0.026	0.012
77949	Centerville		14.1	0.140		0.030	0.025	0.049	0.029	0.008
77952	Provo	River bottom loam	17.0	0.210		0.060	0.024	0.066	0.022	0.011
77957	North Ogden	Heavy loam	13.3	0.180	0.014	0.050	0.017	0.056	0.024	0.014
77959	Honeyville		16.7	0.188	0.014	0.056	0.035	0.060	0.031	0.009
77961	Garland		26.0	0.412	0.028	0.048	0.043	0.080	0.025	0.070
Averages			20.9	0.218	0.032	0.048	0.023	0.062	0.027	0.019
77943	Between Tooele and Grantsville	Alkali (Uncultivated)	64.5	0.216		0.070	0.014	0.071	0.024	0.012
77941	About 1 mi. W. of S. L. C. gas plant		33.2	0.198		0.042	0.012	0.072	0.004	0.021
Averages			48.8	0.207		0.056	0.013	0.071	0.014	0.016

* Soil no. 77954 had a clay substratum.

that it has in tap water. The solubility is shown to be roughly proportional to the percentage of soluble salts in the soil. Thus the third group of soils has the lowest per cent of soluble salts and gives the lowest solubility for the lead arsenate. The second group of soils having the next higher per cent of soluble salts has the next higher solvent power for the arsenate. The fourth group stands next in order in both soluble salts and solvent power, and the College Farm soil follows the fourth group in amount of soluble salts and in solvent power for the arsenate. The two "alkali" soils in the last group break this regularity. These show a somewhat high solvent power for lead arsenate, but the soluble salts in the soils are not shown to be high. In fact, the low per cent of soluble salts found for these two soils is somewhat unexpected, but repetition of the analysis failed to change the result. It is hardly possible to trace any relation between the amount of arsenic dissolved and the individual components of the soil solution, although increase of the carbonate and bicarbonate ions and of the potassium ion is accompanied by increased solution of the arsenate. The fourth group, which contains more chlorides than the second and third, also dissolved more arsenic. These results are in keeping with the finding of Greaves (2).

SUMMARY

Lead arsenate is a very insoluble campound. Only three parts of arsenic dissolve in a million parts of pure water. Its solubility is greatly increased by many common salts when these are present in the aqueous solvent, but sulfates and nitrates do not seem to increase materially the solvent power of water for lead arsenate. Acid salts and those which hydrolize with an alkaline reaction markedly increase the solvent action of water on lead arsenate. The soil solution also has a greater solvent power for lead arsenate than has pure water.

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SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 2

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A POT EXPERIMENT

The general plan of the investigation of which this paper reports a part was outlined in Part I by the senior author. This paper reports data obtained in one of the small-scale pot experiments carried out during the summer of 1914.

Description of the experiment

Five species of plants were used in this experiment, viz., pea, radish, wheat, potato and bean. The soil used was a gravelly bench loam taken from the College Farm. The gravel was removed by sifting. The water-soluble material in this soil amounted to 0.216 per cent. Into each of the 3 gallon, glazed, stoneware jars used in the experiment, a definite weight of soil was placed. This varied from 7000 to 9000 gm. After the crop had been planted, a mulch of 500 gm. of sand was added to each jar. The moisture in the soil used was determined, so the actual dry weight of the soil in each jar was known.

Ten jars were used for each plant studied. These were divided into five sets of two jars each. One set to which no arsenic was added was used for the control plants. During the course of the experiment disodium arsenate was added to the other four sets in varying amounts. Each of the five kinds of plants received the same treatment.

There was some delay in getting the experiment started so the planting did not occur until the middle of June. In some cases the plants did not come up well which necessitated replanting at a later date. This was the case with the wheat, and one or more of the pots had to be replanted for each kind of plant. In particular the radishes were planted very late in the season. This was because lettuce which had been planted two or three times in these pots had failed to grow. Radishes were then substituted for this crop. Some of the potatoes failed to grow at all, and on account of the lateness of the season attempts to get plants in all the potato pots were abandoned. None of the plants, therefore, had arrived at maturity when the experiment was closed on September 30.

TABLE 1

Weight of plants produced and parts of arsenic absorbed per million parts of dry plant

POT NUMBER	APPEARANCE ABOVE GROUND	ARSENIC TREATMENT		AVERAGE AMOUNT OF ARSENIC ABSORBED BY EACH PLANT ON BASIS OF DRY WEIGHT	TOTAL WEIGHT OF DRY PLANTS	AVERAGE WEIGHT PER PLANT
		Commenced	Amount added on basis of dry soil			
			Peas			
			p. p. m.	*p. p. m.*	*gm.*	*gm.*
1 and 2	July 30		0.0	0.0	18.2116	1.1318
3 and 4	30	Aug. 19	25.0	98.0	14.8257	1.4826
5 and 6	30	19	75.0	193.0	16.2201	0.9541
7 and 8	30	19	250.0	1190.0	4.0407	0.8081
9 and 10	*	*	500.0	2150.0	1.7503	0.1591
			Radishes			
11 and 12	Aug. 18		0.0	0.0	22.8076	.....
13 and 14	18	Sept. 2	25.0	53.0	26.0506	.....
15 and 16	18	2	75.0	148.0	30.3656	.....
17 and 18	18	2	150.0	448.0	25.4080	.....
19 and 20	18	2	500.0	940.0	19.6671	.....
			Wheat			
21 and 22	July 25		0.0	0.0	18.867	0.5391
23 and 24	25	Aug. 19	25.0	21.0	22.8928	0.7894
25 and 26	25	19	75.0	52.0	19.7736	0.3954
27 and 28	25	19	250.0	269.0	12.1449	0.4048
29 and 30	25	19	500.0	.....	not weighed	not weighed
			Potatoes			
31 and 32	June 24		0.0	0.0	9.1891	9.1819
33 and 34	25	Aug. 19	25.0	78.0	27.7595	6.9399
35 and 36	24	19	75.0	347.0	3.2245	1.6122
37 and 38	28	19	250.0	524.0	7.9895	7.9895
39 and 40	crop failed					
			Beans			
41 and 42	July 11	Aug. 19	0.0	Trace	14.4565	2.8911
43 and 44	28	19	25.0	50.0	11.9991	1.9998
45 and 46	26	19	75.0	155.0	7.9218	1.1317
47 and 48	26	19	250.0	678.0	7.2346	1.8086
49 and 50	19	19	500.0	1965.0	2.3626	0.4725

* Plants appeared above ground Aug. 24 in pot 9 and July 30 in pot 10; arsenic was added to pot 9 on Sept. 2, to pot 10 on Aug. 19.

Since the age of the plant might affect its power to withstand the poison, it was intended to allow the plant to get a pretty substantial growth before the treatment with arsenic was commenced. The plants were, therefore, allowed to grow as long as possible before adding any arsenic to the soil. The length of time intervening between the appearance of the plants above the soil and commencement of the arsenical treatment varied from three to six weeks, the dates in each case are given in the table.

During the experiment the moisture content of the soil was kept at approximately 20 per cent, by adding water at the surface three times a week. When the treatment with arsenical solution commenced the disodium arsenate solution was added with the irrigation water, in nine equal dosages. It required, therefore, three weeks to add all the arsenic used in the tests. In those cases in which the arsenical treatment commenced on August 19 the arsenic was all added by September 8. In these cases, therefore, a growing period of three weeks intervened between the end of the arsenical treatment and the end of the experiment.

At the close of the experiment of the plants, including roots, stems and foliage were harvested. They were dried, weighed and analyzed for arsenic. In making the analyses, all the plants produced in one jar were taken together. The adhering particles of soil were washed from the roots with distilled water, so the arsenic found in the analysis was what had actually been absorbed by the plants. The method of destroying organic matter and obtaining the arsenic in solution used in this work is described by Fresenius (3). The Marsh method, as modified by Greaves (5), was used for determining the arsenic. Results are given in table 1.

Discussion of data

The conclusions to be drawn from a preliminary and brief experiment of this kind are mainly of a tentative character, except possibly as the results may serve to corroborate earlier observations. The fact that arsenical compounds are poisonous to plants has been established, and this experiment corroborates that conclusion. But there is not a great deal of evidence in the literature showing that higher plants are stimulated to more vigorous growth by arsenical compounds.

At Rothamsted, water cultures by Brenchley (1) in very dilute sodium arsenate solutions 0.004 part per million as the lower limit), containing the essential nutrients, failed to show any stimulation of peas or barley. However, at that station, barley grown in nutrient solutions containing 1.0 to 0.05 part of arsenious acid per million looked as if stimulation had occurred, but the dry weights of the plants did not support that conclusion. Knop (7), in 1884, found that 50 parts of arsenic acid per million of nutrient solution did not check the growth of a strongly rooted maize plant which was transferred to the solution containing arsenic acid. There seems to have been very few experiments made up to date to show the effect of arsenic compounds on plants when grown in their natural habitat, the soil. Statements made by some of the earlier investigators in this line seem somewhat remarkable in the light of present knowledge. For instance, Davy (2), in 1859, and Gorup-Besanez (4), in 1863, agree in the

statement that arsenious acid is without effect on peas grown in the soil. But at Rothamsted (1) peas were found to be rather sensitive to arsenical poisoning; and we have found, as herein reported, that peas are quite sensitive to the presence of disodium arsenate in the soil. Disodium arsenate is now well known to be a less energetic form of the poison than the arsenious acid used by Davy and Gorup-Besanez.

So the literature at the present time indicates that it is very doubtful whether or not compounds of arsenic are able to stimulate the growth of the higher plants, although it is now well known that certain molds and other lower plants are stimulated by, and grow luxuriantly in the presence of large quantities of arsenic compounds. Greaves (6) reported a stimulation of nitrifiers and ammonifiers in the presence of several arsenic compounds.

It seems reasonable to expect to find such a stimulation in the higher plants, but what are we to use as a criterion of such stimulation? If stimulation occurs will the dry weight of the plant necessarily be greater than it otherwise would have been? Is not a healthy, vigorous appearance also evidence of beneficial influence? The life processes of a healthy looking plant may proceed more rapidly than those of a plant of less vigorous appearance, and the former may arrive at maturity at a somewhat earlier date than the latter without attaining a greater weight.

Our experiment seems to indicate that disodium arsenate in the lower concentrations had a beneficial influence on the plants in all the cases. So far as appearances can be relied on, beans, potatoes, peas and wheat seemed more vigorous and healthy in the pots containing 25 parts of arsenic per million of soil than they did in the control pots, and in the case of peas and wheat the plants in the pots containing 75 parts of arsenic also seemed to be stimulated. this healthy appearance in the case of wheat especially, and to a less degree in the case of beans, is shown in plate 1. The photographs show the effect of the arsenic in producing apparent stimulation in the lower concentrations and checking growth and causing death in the higher concentrations. The dry weights of these plants, as shown in table 1, however, do not give definite evidence of having been increased by the arsenic in the lower concentrations, although they show plainly a decrease for the higher concentrations. Unfortunately, the value of the dry weight data is not as great as it would have been if there had been the same number of plants in each pot.

The radishes, on the other hand, gave no visible evidence in the foliage of the effect of the arsenic. But when the crop was harvested at the end of the experiment the parts of the plants below ground showed plainly the effects of the different treatments. The radishes grown in the pots of low arsenic content had thick fleshy, fine looking roots, while those grown in the pots of high arsenic content were much longer and of smaller diameter. In this case the dry weights shown in table 1 seem to indicate rather decidedly that the arsenic had had a stimulating influence up to and including the concentration of 250 parts of arsenic per million of soil. The radish plants were not counted, but the stand seemed very uniform in all the pots.

Our data indicate that the plants used are not equally resistant to the effect of the arsenical poisoning. This accords with the observations of others

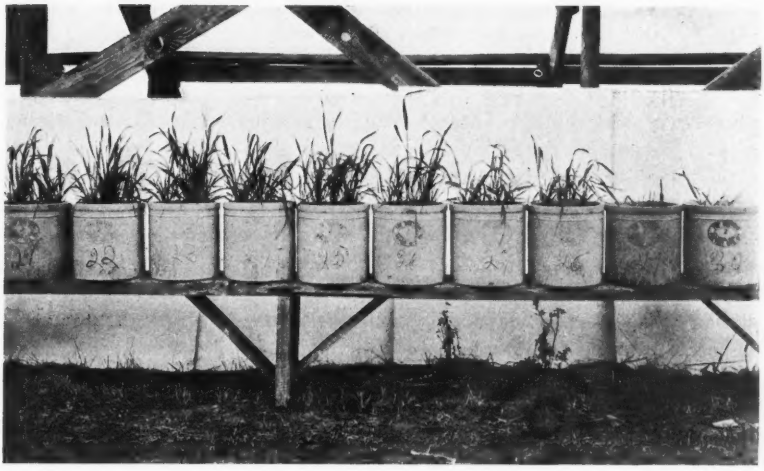

FIG. 1. WHEAT GROWING ON SOIL TREATED WITH 0 TO 500 PARTS OF SOLUBLE ARSENIC PER MILLION PARTS OF DRY SOIL

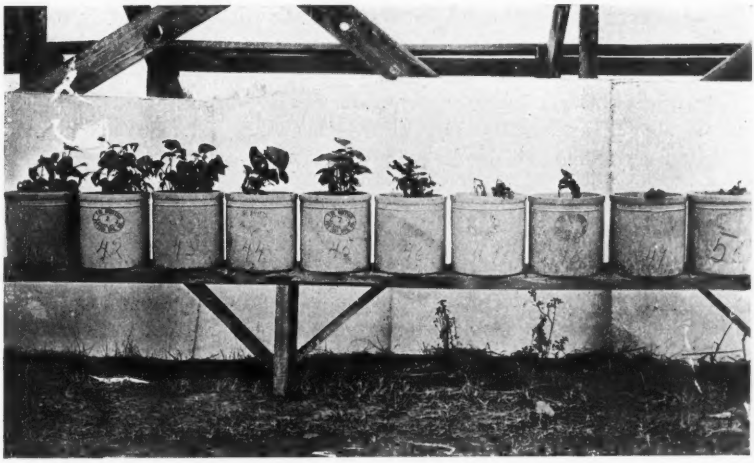

FIG. 2. BEANS GROWING ON SOIL TREATED WITH 0 TO 500 PARTS OF SOLUBLE ARSENIC PER MILLION PARTS OF DRY SOIL

to the effect that the specific nature of the plant is an important factor in its behavior toward poisons. Beans seemed decidedly more sensitive to the poison than the other plants. Those in pots 49 and 50 were dead on August 25 after only two treatments with arsenic. On September 8 at the close of the arsenical treatment the beans in pots 47 and 48 were turning yellow and appeared very sick, while those in pots 45 and 46 had some yellow leaves. Beans of good size were produced by the plants in pots 41 to 44, inclusive, but in pots 45 and 46 only small, immature beans were produced. Potatoes seemed a close second to beans in sensitiveness. The plant in pot 37 was a large healthy one before treatment with arsenic, but it soon showed the yellow withering of the lower leaves. The plants in pots 33 and 34 were the only ones that produced tubers, and these measured 1 to $\frac{1}{2}$ inch in diameter. Peas were more resistant than beans and potatoes and seemed more sensitive than wheat. In pots 9 and 10 the peas commenced to wilt and turn yellow in the lower leaves after three treatments with arsenic. They died after six treatments. Radishes seemed decidedly more resistant than the other plants tested, although those in pot 20 died after five arsenical treatments. Still, those in pot 19 undergoing duplicate treatment flourished and made a better yield than either of the control pots, 11 or 12.

The amount of arsenic absorbed by the plant which is necessary for the checking of growth seems to have been, in parts per million of dry plant matter, 50 for beans, 78 for potatoes, 52 for wheat, 193 for peas, and 940 for radishes. When the plant is killed the smallest amount of arsenic absorbed was shown to be 269 parts per million for wheat, 524 for potatoes, 678 for beans, 1190 for peas, 940 for radishes.

The wheat was not killed entirely even in the pots containing the most arsenic, 500 parts per million parts of dry soil, and, in the two pots containing 250 parts per million, the plants made a decided recovery after the arsenical treatment had ended on Sept. 8. While the wheat seemed more resistant to the arsenic in the soil than did the other plants except radishes, it had absorbed in each case less arsenic in proportion to its dry weight. Its apparently greater power of resistance may thus be due to a difference in root activity rather than to the power of the plant to live with more or less arsenic in its tissues. We were unable to determine the arsenic actually absorbed by the wheat in the pots treated with 500 parts of arsenic per million of soil on account of the fact that the dead parts of the plants had become soaked with the unabsorbed arsenic in the soil during a rainstorm. This is the reason also for the high amount of arsenic reported in the radishes in pot no. 20. The value of the data showing the amount of arsenic absorbed by the wheat and the weight of the wheat plants is, however, somewhat marred, though not completely destroyed by the fact that a horse grazing on the campus ate off the tops of all the wheat plants after September 25th.

Is the fine, healthy appearance of plants grown in the presence of small quantities of arsenic compounds due to the destruction, by the arsenic, of soil

microörganisms which are injurious to the higher plants or is it due to a direct action of the arsenic compound on the higher plant? While in water cultures injurious microörganisms are not necessarily excluded, still the fact that this healthy appearance of the plants develops in these cultures tends to show that it is due to a direct chemical-physical action. But both kinds of action may be involved. The problem is interesting and awaiting solution.

In our experiment, as in those of others already reported, and in the literature, the effect of arsenical poisoning of higher plants is seen to be a destruction of the chlorophyll with accompanying death of the leaves affected. This yellowing of the leaves commences at the lower ones and gradually extends up the plant. This indicates that the poisoning is probably due to a chemical action on the chlorophyll in which the arsenic is involved. If the poisonous action were due in the main to injury to the root or tissues of the stem, its visible effects should be first seen at the top of the plant and extreme tips of the leaves, which is exactly the opposite of what is actually observed.

In this experiment it appears that 75 parts of arsenic per million of soil is not injurious to some plants, while the more sensitive ones are slightly affected, but 25 parts appear to be stimulating. These quantities are equivalent to about 375 and 125 parts per million of soil solution. In the previous paper (Part 1) by the senior author, it was shown that lead arsenate is soluble in the soil solution to the extent of about 10 to 64 parts of arsenic per million of soil solution. These results indicate tentatively, at least, that spraying orchards with lead arsenate may safely be continued for a number of years from the beginning of the spraying.

SUMMARY

Our results give visible evidence of stimulation, or beneficial influence, of disodium arsenate in low concentration on all the plants tested. In the case of the radish, however, this visible result appears in the underground part and not in the foliage. The evidence of stimulation or non-stimulation shown by the dry weights is inconclusive, but indicative of stimulation where the concentration of the arsenate was low, and in the case of the radish in all the concentrations tested. It would, therefore, seem that the accumulation of arsenic in the soil, as a result of the spraying of orchards, if not continued to excess, may be beneficial rather than injurious. A thorough study of the questions involved herein would probably lead to interesting and important results and conclusions in relation to the problems of disease resistant plants and of immunity in general; and, since the chlorophyll is involved in the poisoning, the problem evidently includes the very fundamentals of plant growth.

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NITROGEN FIXATION IN ARID CLIMATES

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In a country such as the Punjab, the soil receives very small applications of manure. It is estimated that in a typical irrigated colony tract, the land receives on the average not more than between one-half to one ton of farm yard manure each year. In non-irrigated tracts (so called *barani* land) it may be taken that no manure is added to the soil since any manure available is applied to well irrigated land in the neighborhood. In some tracts it has been the custom to grow wheat after wheat with no application of manure at all. The economic conditions and the conservatism of the peasantry make any general use of commercial organic manures, such as cake, a remote possibility for a long time to come. Any possibility of bringing under control the factors which determine the natural processes of nitrogen fixation which must be taking place in the soil, is therefore of enormous importance.

That the natural recuperative powers of soils in arid regions must be considerably greater than those met with in more temperate climates, is shown by crop yields obtained when moisture conditions are favorable. Yields of wheat, which would certainly not be expected from a similar soil in a temperate region, are obtained on what would be regarded, from its history, as totally exhausted land. It would seem justifiable to suppose therefore that the climate may have a large influence on the activity of free-living nitrogen-fixing organisms. Moreover, it is possible that the frequent cultivation which the tradition of the peasant leads him to give his soils during the hot-weather fallow between his wheat crops, may not only conserve moisture but also bring about suitable conditions for active nitrogen fixation.

There can be no doubt that in *barani* tracts agriculture is almost wholly dependent on the natural processes of nitrogen fixation; that these processes are of almost equal importance under the better conditions of agriculture possible in irrigated tracts may be seen from the following considerations. Table 1 gives the area under various crops in the Lower Chenaab Canal colony, together with a conservative estimate of their average yield. The amount of nitrogen in the total produce is then calculated, largely on the basis of the analyses of Sen (1). This figure shows, on comparison with the average application of farm yard manure, that artificial sources can only account at most for about one-sixth of the total produce. Otherwise expressed, natural processes of fixation add something in the neighborhood of 38 pounds of combined nitrogen per acre per year.

TABLE 1

Estimate of average annual loss of nitrogen by cropping

CROP	AREA SOWN	YIELD PER ACRE		TOTAL NITROGEN CONTENT		TOTAL NITROGEN CONTENT OF CROP	
		Grain	Straw	Grain	Straw	Grain	Straw
	acres	*maunds**	*maunds*	*per cent*	*per cent*	*maunds*	*maunds*
Wheat	986,591	16	35	1.50	0.5	236,800	172,700
Barley	17,751	12	20	1.49	0.6	3,175	2,130
Rice	32,075	20	30	1.01	0.6	6,480	5,774
Maize	103,197	22	60	1.34	2.0	30,420	123,836
Mixed grain	10,509	23	35	2.68	0.7	6,479	2,575
Great Millet (*Jowar*)	16,071	8	60	1.40	0.6	1,800	5,785
Spiked Millet (*Bajra*)	35,092	9	60	1.86	4.7	5,874	98,960
Italian Millet (*Kangni*)	377	8	20	1.91	1.8	57	135
Gram	55,578	12	30	3.06	0.7	20,490	11,671
Lentils, etc	6,317	8	15	3.94	0.8	1,991	748
Cotton	315,067	5	60	2.82	0.5	44,430	94,520
Til	777	10	30	3.62	0.5	281	116
Sarson, Toria (*Brassica Compestris*)	236,045	7	25	3.30	2.6	54,540	135,400
Linseed	1,200	6	8	3.19	0.5	229	48
Sugar Cane	59,443	300		0.19		33,890	
Fodder	378,607	200		0.8 (Dry)		181,731	
Totals	2,258,328					633,667	672,407

	maunds
Total nitrogen removed	1,306,074
Nitrogen added as manure, estimated at 21 maunds of farm yard manure per acre analysing at 0.475 per cent nitrogen	225,268
Difference equivalent to about 38 lbs. nitrogen per acre	1,080,806

* One maund = about 82 lbs.

TABLE 2

Nitrogen per 100 grams in lyallpur soils on dates shown

DATE OF SAMPLING	SANDY LOAM	LOAM	CLAY LOAM
	mgm.	*mgm.*	*mgm.*
May 15, 1916	22.8	39.4	51.0
June 13, 1916	31.0	41.0	62.0
July 1, 1916	29.0	48.0	54.0
July 17, 1916	29.0	48.0	56.0
August 15, 1916	25.0	41.0	49.0
September 18, 1916	39.0	53.0	67.0
December 1, 1916	127.0	83.0	97.0
Land prepared and wheat sown			
March 14, 1917	70.0	84.0	69.0
Wheat growing; harvesting to be done after a month			

In the year 1916 an extended series of field analyses were initiated at Lyallpur by the late J. H. Barnes (2). These were continued and supplemented with laboratory investigations by the present authors in succeeding years. In 1916 most remarkable results were obtained. The average fixation in four different districts of the Punjab amounted on the average to more than 100 per cent of the total nitrogen in the soil. The possibility of any consistent error is precluded by the results of a more detailed examination of the soils of Lyallpur where the nitrogen content of the soil was estimated at frequent intervals between the wheat harvest in April and the date of sowing in the following November. The results are reproduced in table 2 and plotted together with the rainfall in figure 1. It will be seen that with all three soils examined, after a preliminary period of depression during the rains, rapid

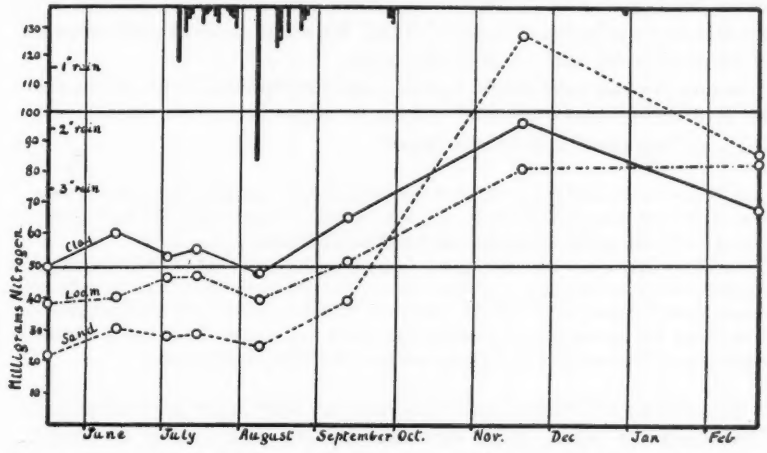

FIG. 1. RELATION OF RAINFALL AND FLUCTUATIONS IN NITROGEN CONTENT OF LYALLPUR SOILS

increase took place during September and October. The astounding magnitude of the fixation may be realized by considering that an addition of 50 mgm. of nitrogen per 100 gm. of soil (the average fixation in 1916) corresponds with an application of about 150 tons of farmyard manure to the acre.

In 1917 experiments were continued on a smaller scale but failed to indicate any fixation. A more extended series of analyses were made in succeeding years, great attention being paid to accuracy of sampling, and the limits of the experimental error. The results will be found in detail in the Report of the Agricultural Chemist, (loc. cit.) for 1918 and succeeding years, and can only be summarized here. As a result of a calculation of the probable field and laboratory errors it was decided to disregard as evidence of either fixation or denitrification any changes smaller than 8 per cent of the total nitrogen content of the soil. The results of the large number of observations made are

difficult to summarize concisely. The results are also extremely variable on account of differences of climate and cultivation. In table 3 the soils have been divided into two classes, those in which the rainfall is above 10 inches and below 10 inches. These classes are again subdivided according to the treatment received by the soil. It will be seen that in no case during the succeeding years do the results approach in magnitude those obtained in 1916. Although isolated instances of considerable fixations occur, there is no uniformity. It is also impossible to correlate the detailed results with either rainfall, cultural, or temperature factors. By the collection of more evidence this may ultimately become possible; for the present we must recognize that peculiarly favorable conditions must have prevailed in 1916. One cannot help comparing the sudden activity of the nitrogen-fixing organisms in the soil of the Punjab with the increase of virulence of pathogenic organisms as in the case of the influenza epidemic of 1919. We are at present equally ignorant of the predisposing causes in both these cases.

In order to gain some insight into the factors controlling fixation in the field, in 1919 an elaborate series of laboratory experiments was started. The following hypotheses may be entertained:

1. It may be assumed that if the great heat of the Punjab summer causes partial sterilization of the soil, when brought under optimum conditions, azotabacter will have a better chance to develop rapidly in the absence of predatory protozoa.
2. Azotobacter alone may not be the determining factor; suitable conditions may be requisite for the development of another agent either previously to, or together with, the development of azotobacter. Thus it is well known that the presence of carbohydrate food is necessary for the functioning of azotobacter, and it has been suggested that a symbiotic relationship exists between it and species of algae which develop in the soil.

In order to test these hypotheses, samples were taken at various dates throughout the fallow, which were then brought under, as far as possible, optimum conditions in the laboratory and the rate of change of nitrogen content determined. In order to test the possibility of partial sterilization in the field, the plots from which samples of the two types of soil examined were taken were duplicated, one receiving normal fallow treatment (cultivation after rain), the other being kept repeatedly stirred. The samples thus taken were brought to their optimum moisture content and incubated under three separate conditions until the following November. A sufficient number of separate samples of the main samples taken each month were set aside in order to provide the necessary duplicates for analysis each succeeding month. Of the three series of samples, one was incubated in the laboratory in diffused light, and another in a dark incubator. It was thought that any differences observed in the rate of nitrogen fixation in these two series might be due to the fact that algae would be unable to develop in the dark. The third series was placed in large earthenware pots sunk flush with the ground out of doors, but protected from rain and dust by glass plates.

TABLE 3

Abstract of nitrogen-fixation results, expressed as percentages of original nitrogen contents of soil, and classified according to year, rainfall, cultivation

(Figures with a negative sign prefixed represent losses in total nitrogen)

CLASSIFICATION OF SOILS	1916				1917				1918				1919				1920			
	Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen		
		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average
		per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
Rainfall, 0–10 inches																				
Cultivated	3	413.0	90.2	204.7	3	−36.4	−4.5	−23.2	22	21.4	−12.9	−0.3	10	9.7	−12.4	−0.5	6	8.7	−14.2	−2.8
Uncultivated													9	10.7	−8.6	0.7	5	9.5	−11.6	−1.1
Rainfall, 10–15 inches																				
Cultivated	6	199.0	66.7	132.4					9	30.9	−21.8	*±0.0	6	3.4	−5.7	−1.1	1		−1.7	−1.7
Uncultivated													7	13.1	−11.9	+0.2				

* Would seem to be 4.5 per cent.—ED.

The most direct method of testing the hypotheses would no doubt be to supplement experiments similar to those described above by enumeration of the number of azotobacter, protozoa and algae. The technical difficulties involved are however very great, so it was decided in the preliminary experiments to rely solely on the chemical evidence. Qualitative results were obtained as to the prevalence of protozoa, but the evidence was not sufficiently reliable to enable any conclusions to be based on it. It is hoped that this aspect of the work will be developed in the future.

The largest fixation observed was not more than 45 per cent, showing that we had not been successful in reproducing the favorable conditions of 1916. The results were, moreover, very variable, due probably to the difficulty of keeping the soil of small samples at optimum moisture content for long periods and at the same time avoiding spoiling the texture. It is possible however to draw certain preliminary conclusions. The most marked and uniform fixation with all soils and under all conditions of incubation took place in September. This is the period at which the rapid increase took place in the field in 1916. Thus of 12 samples the average fixation was 15.5 per cent, and with one soil about 40 per cent. At no other date was there a consistent fixation with all samples. With the samples isolated in May and incubated till the following October, no definite increase was observed which was far outside the limits of the allowable error. The general course of events however appears to be similar, both with the different soils, and different conditions of incubation, as will be seen from table 4.

It seems legitimate to conclude, therefore, that the date of sampling is of the utmost importance in estimating the nitrogen-fixing powers of a soil in the laboratory. There appears to be a definite seasonal influence which must be taken into account.

The results so far obtained fail to enable us to form an opinion as to the effect of the partial sterilization possible in the soil under ordinary cultural conditions. In some cases the fixation was greater in the soil taken from the pulverized plots, and sometimes the reverse. The experiment was however conclusive in showing that it is not till after a prolonged period of dry heat that the soil becomes capable of considerable nitrogen fixation. Much more data must be made available before we can hope successfully to correlate the results of chemical and biological examination with the seasonal influences. Our present knowledge appears to indicate that it is the seasonal influence which is of primary importance. It may therefore be necessary to await the passage of many seasons before it will be possible to discover all the factors which control nitrogen fixation in the soil. As in the case of influenza, the endemic activities of the soil organisms may afford much valuable information but it may be necessary to wait for the next epidemic before we shall be able to solve the problem of their sudden virulence.

Another aspect of the question which remains to be studied is seen if we consider that in order to utilize the nitrogen fixed in the soil, it must be sub-

sequently nitrified. From the results obtained it appears that a rapid period of nitrogen fixation is followed by an almost equally rapid loss. If, however, it is found possible to control the nitrification of only a small fraction of the amounts of nitrogenous organic material which may be synthesized in the soil, we shall have travelled a long way in the direction of making the soil self-supporting in its nitrogen economy.

Almost the whole of the laboratory work referred to in this paper has been carried out by Mr. Barkat Ali. Acknowledgment must also be made to Mr. S. M. Nasir for much painstaking assistance.

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OXIDATION OF IRON PYRITES BY SULFUR-OXIDIZING ORGANISMS AND THEIR USE FOR MAKING MINERAL PHOSPHATES AVAILABLE[1]

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INTRODUCTION

It is very well known among sulfuric acid manufacturers and others that iron sulfides (pyrites) tend to oxidize slowly into sulfates. These changes seem to be more rapid when the pyrites are moist or lying on the gound. They first oxidize, forming ferrous sulfate, and since this compound is not stable in presence of water or moist air, this is converted into basic iron sulfates and then into iron hyroxides which are but slightly soluble.

Löhnis (3) describing the influence of microörganisms on the sulfur cycle (p. 705) says that they do the principal work. The sulfides are transformed into hydrogen sulfide; the same may happen with the thiosulfates and more seldom with the sulfates. The H_2S and the thiosulfate are oxidized into sulfates. At another place, however, (p. 708) he lays emphasis on the fact that the oxygen of the air has a strong oxidizing action. Allen and Johnston (1) found that when pyrites are ground for analyses they suffer partial oxidation to sulfur dioxide and ferrous sulfate. Whether or not this action is of a purely chemical nature Kappen and Quensell (5) have tried to ascertain. They weighed into a porcelain dish, in the bottom of which was a filter paper, 100 grams of sandy soil, moistened with 15 cc. of water, and drained off the surplus with a suction pump. Hydrogen sulfide was passed through the soil till the mass had a black color. The soil was then placed in the open air and in 30 minutes the color had changed and the iron sulfide changed into iron sulfates. After standing for 5 hours the soil was treated with 10 per cent HCl and the amount of sulfuric acid determined in the extract. For 100 grams of untreated soil they found as an average 2.11 mgm. of sulfur as sulfates at the beginning and 2.34 mgm. after 5 hours. With the treated soil they found 4.15 mgm. at the beginning and 5.01 mgm. after 5 hours. They draw the conclusion: "dass überall da, wo sich im boden durch Faulnis von Eiweisstoffen oder durch Reduktion von Sulfaten Schwefelwasserstoff bildet, oder sofort durch dass wohl in allen Böden in dazu genügenden Mengen vorhandene Eisenoxydhydrat unter Schwefelabscheidung und Reduktion des Eisenoxydes gebunden wird, und dass das Schwefeleisen, die Zutritts-

[1] Paper No. 91 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in Rutgers College Studies, Vol. 1.

[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This work was started in France and partially repeated at the New Jersey Agricultural Experiment Stations.

möglichkeit von Sauerstoff natürlich vorausgesetzt, sich ohne Einwirkung von Mikroorganismen weiterzersetzt. Die höchste Oxydationsstoffe des Schwefels, die wieder ohne weiteres in den Kreislauf eintreten könnte, entsteht hierbei nicht, oder nur in spuren; es bildet sich vielmehr hierbei fast ausschiesslich elementarer Schwefel." Their further studies led them to conclude that this elementary sulfur is changed to sulfates by various influences. Van Bemmelen (7, p. 81, 85, 97, 98) pointed out that pyrites occur locally in diatoms and also in plant cells. He explains the formation of pyrites as a sulfate reduction. The iron in the soil is changed into iron sulfates in the presence of sulfates. By the oxidation of the iron sulfite sulfuric acid is formed. This sulfuric acid does not attack, or but very slightly attacks the silicates in the soil, but does attack the iron oxide present. This investigator concludes (p. 97) "that the iron oxide in the soil protects, to a certain extent, the humate and silicate bases against the action of the sulfuric acid." Iron sulfate is formed from this acid and the iron after the oxidation of the sulfur to sulfuric acid.

The studies made by the writer upon this subject are in general accordance with Van Bemmelen's conclusions, but so far as biological factors are concerned, considerable room for study is left.

PURPOSE OF INVESTIGATION

From the work of Kappen and Quensel, Allen and Johnston and others it would seem that the action of bacteria on the transformation of iron sulfide into iron sulfate is slight or negligible.

Two main points were open for investigation:

A. Do sulfur oxidizing organisms transform iron sulfide into iron sulfate?

B. If so, what is the influence of this transformation on the availability of rock phosphate composted with a mixture of pyrites, sulfur and soil?

EXPERIMENTAL RESULTS

Series 1

A slightly acid air-dry soil was secured and mixed thoroughly with not very finely ground iron pyrites. This mixture was divided into two parts of which one part was inoculated with a soil-compost extract known to contain sulfur-oxidizing organisms. An equal amount of a slightly acid air-dry soil was mixed with flowers of sulfur, divided, and one part treated in a similar way as is described above. The mixture contained 50 parts of soil and 100 parts of sulfur, while the amounts of pyrites used were calculated to contain the same quantities of sulfur. The iron pyrites consisted of 45.6 per cent of sulfur and 47.8 per cent of iron. The water-holding capacity of the mixtures was dedetermined according to the Hilgard method and the moisture content kept at the supposed optimum of 60 per cent of the water-holding capacity by adding twice a week the amounts of water lost. The triplicate mixtures were placed in Erlenmeyer flasks closed with cotton plugs, stirred once every two weeks and kept at 28°C. during the incubation period of 14 weeks. Determinations of hydrogen-ion concentration, relative acidity and water-soluble sulfates were made at intervals of two weeks. The relative acidity and hy-

drogen-ion concentrations were determined in the manner described in an earlier paper (10). The water soluble sulfates were determined in the following way:

The composts were thoroughly mixed and 4 grams transferred to a 500-cc. flask with about 200 cc. distilled water and 5 cc. HCl (22°B). The flasks were vigorously shaken and left standing for 12 hours; filtered into flasks of 250-cc. capacity, an aliquot drawn off, and precipitated with barium chloride. The precipitate was filtered off, washed and calcined, dried and weighed as barium sulfate.

The data secured are given in table 1.

TABLE 1

Comparative effect of inoculation on the production of water-soluble sulfates in mixtures of soil and iron pyrites and of soil and sulfur

PERIOD OF INCUBATION	SOIL AND IRON PYRITES COMPOST							SOIL AND SULFUR COMPOST						
	Not inoculated			Inoculated				Not inoculated			Inoculated			
	Reaction		Soluble sulfate	Reaction		Soluble sulfate	Increase over check	Reaction		Soluble sulfate	Reaction		Soluble sulfate	Increase over check
weeks	*pH*	*cc.**	*per cent*†	*pH*	*cc.*	*per cent*	*per cent*	*pH*	*cc.*	*per cent*	*pH*	*cc.*	*per cent*	*per cent*
0	6.1	8.9	0.63	6.1	8.9	0.63	....	4.6	23.4	0.13	4.6	23.4	0.13	....
2	6.0	9.2	0.88	5.7	14.2	1.96	1.08	4.5	24.8	0.12	3.9	36.1	0.88	0.66
4	6.0	9.4	0.97	4.4	16.2	2.47	1.50	4.7	26.2	0.16	2.6	172.1	2.32	1.16
8	5.9	9.7	1.12	3.6	20.5	3.27	2.15	4.4	25.8	0.15	2.1	279.5	2.82	2.67
12	5.8	10.9	1.26	3.9	28.3	4.12	2.86	4.4	26.0	0.19	2.0	322.2	3.22	3.03
14	5.7	12.2	1.38	4.2	47.5	4.65	3.27	4.4	27.2	0.21	2.1	305.9	3.66	3.45

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.
Total sulfur content was taken as 100%.

It is evident that, under these conditions, oxidation of iron pyrites took place in the uninoculated mixtures, as is indicated in the change of hydrogen-ion concentration, the increase in relative acidity and the per cent of water-soluble sulfates formed after 14 weeks. The increase was gradual, as was the case in the inoculated mixture. However, the change in pH values in the inoculated mixtures of soil and iron pyrites was considerably greater, and the relative acidity increased more rapidly. The water-soluble sulfates formed had increased at the end of 14 weeks to more than 3 times the sulfates formed in the uninoculated mixture. The total water-soluble sulfates of the uninoculated mixtures after 14 weeks was but 1.38 per cent of the sulfur present, while the total water-soluble sulfates of the inoculated mixtures after the same time of incubation had increased to 4.65 per cent of the sulfur present. The mixtures were less frequently aerated in order to see whether or not the inoculated mixture would produce more sulfates under the circumstances than the uninoculated pyrite-soil mixture.

The pH values in the inoculated mixtures went down to 3.6 after 8 weeks and from then on went up again, undoubtedly because the action of the acid upon the pyrites constitutes a buffer action.

In the uninoculated soil and sulfur mixtures the pH value went down gradually also, accompanied by a slight increase in relative acidity and water-soluble sulfates. It should be stated here that it was difficult to keep the uninoculated mixtures free from contamination. It is possible therefore that after some time the uninoculated mixtures contained sulfur-oxidizing organisms. In view of this possibility the cultures were discarded after 14 weeks. The inoculated sulfur-soil mixtures increased rapidly in acidity as shown by the higher hydrogen-ion concentration and the titrated acidity. The sulfur present was oxidized at nearly the same rate as in the case of the inoculated mixtures of iron pyrites and soil, the former being but 0.18 per cent more at the end of 14 weeks. If the mixtures had been more frequently aerated this difference would have been greater as indicated by other experiments. All mixtures remained closely packed and were not stirred except for taking samples.

As could be expected, the hydrogen-ion concentration was considerably higher in the sulfur mixtures on account of the lack of material, except for the soil constitutents, with which the sulfuric acid might react. No attempt was made, however, to make a careful study of the possible increase in soluble potassium, iron, phosphorus, etc., present in the soil.

A second series conducted in tumblers covered with glass plates gave essentially the same results, but contamination was more evident after 10 weeks.

From the results obtained, the conclusion was drawn that sulfur-oxidizing organisms are active in the transformation of iron sulfides into iron sulfates when the pyrites are mixed with soil and kept at a supposed optimum moisture-content.

Series 2. Pyrites composted with a mixture of soil, sulfur and rock phosphate

As has been pointed out it was interesting to find out what the influence of iron pyrites would be on the availability of rock phosphate when composted with a mixture of soil and sulfur. A series of experiments was conducted in which 100 parts of soil were mixed with 400 parts of rock phosphate and different amounts of sulfur and pyrites. For this purpose a slightly alkaline calcareous soil was used and the sulfur replaced by pyrites so as to have approximately the same amounts of sulfur in all experiments. The exact quantities of pyrites used together with the data secured in 12 weeks are given in table 2. The relative acidity and hydrogen-ion concentration was determined at intervals and the available P_2O_5 determined after 9 and after 12 weeks. One series of cultures was kept at room temperature and another series with the same additions incubated in darkness at 30°C. The pH values for the cultures incubated at 30°C. had after 5 weeks reached the point at which the phosphoric acid becomes available. The relative acidity increased rapidly

until the end of 7 weeks but from then on increased less rapidly. This could be expected on account of the neutralization of the acid by the tricalcium phosphate present. At the end of 9 weeks and at the end of 12 weeks there

TABLE 2

Influence of iron pyrites composted with a mixture of soil, sulfur and rock phosphate on the availability of P_2O_5

NUMBER	MATERIALS IN ADDITION TO PHOSPHATE AND SOIL†	INITIAL REACTION		AFTER 9 WEEKS			AFTER 12 WEEKS		
				Reaction		Soluble P_2O_5	Reaction		Soluble P_2O_5
	Incubated at 30°C.								
		pH	*cc.**	*pH*	*cc.*	*per cent‡*	*pH*	*cc.*	*per cent*
1	90 sulfur, 60 pyrites	6.9	0.0	3.1	178.0	9.39	3.0	198.4	10.62
2	80 sulfur, 80 pyrites	6.9	0.0	3.1	174.0	8.93	3.1	189.8	9.52
3	70 sulfur, 100 pyrites	6.9	0.0	3.3	152.0	8.28	3.1	175.3	9.94
4	60 sulfur, 120 pyrites	6.9	0.0	3.0	171.0	9.20	3.0	189.4	10.62
5	120 sulfur	6.9	0.0	3.2	161.2	8.88	3.0	180.1	9.98
	Incubated at room temperature								
6	90 sulfur, 60 pyrites	6.9	0.0	5.6	7.6	0.00	4.6	48.7	0.76
7	80 sulfur, 80 pyrites	6.9	0.0	5.4	16.5	0.00	4.0	53.2	1.96
8	70 sulfur, 100 pyrites	6.9	0.0	4.9	14.4	0.00	3.7	48.6	2.02
9	60 sulfur, 120 pyrites	6.9	0.0	5.2	10.1	0.00	3.8	21.5	1.88
10	120 sulfur	6.9	0.0	5.0	14.2	0.00	3.7	49.4	1.98

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.
† All mixtures contained 100 parts of soil and 400 parts of rock phosphate.
‡ Total P_2O_5 content was taken as 100 per cent.

was no appreciable difference in total soluble P_2O_5 and acidity in any of these cultures, and they did not appreciably differ from the cultures without pyrites used as checks. The same was true of the cultures kept at room temperature. The influence of temperature was very pronounced in these cultures. In

the cultures incubated at toom temperature but little P_2O_5 had been made soluble after a period of 12 weeks. However, the accumulation of acidity in the mixtures with pyrites was as gradual as in the mixtures without pyrites, being approximately the same in most of the cultures. It seemed therefore that the iron pyrites did not interfere with the formation of sulfates, nor with the availability of phosphoric acid.

Series 3. Substitution of ammonium sulfate for soil

A series of experiments similar to those reported in a previous paper (10) table 5 was conducted with a mixture of 10 parts of soil, 400 parts of rock

TABLE 3

Effect of partial replacement of soil by ammonium sulfate in compost of soil, rock phosphate, sulfur and iron pyrites

NUMBER	TREATMENT†	INITIAL REACTION		REACTION AFTER 2 WEEKS		REACTION AFTER 4 WEEKS		REACTION AFTER 6 WEEKS		REACTION AFTER 10 WEEKS		SOLUBLE P_2O_5	REACTION AFTER 12 WEEKS	
		Incubated at 30°C.												
		*cc.**	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent‡*	*cc.*	*pH*
1	None	0.2	6.9	2.8	6.4	41.2	4.2	74.5	3.8	92.1	3.8	5.12	101.2	4.0
2	0.2 per cent $(NH_2)SO_4$	0.6	6.7	43.5	4.1	76.7	3.8	127.3	3.6	132.2	3.3	9.54	153.9	3.9
		Incubated at room temperature												
3	None	0.2	6.9	1.9	6.6	20.6	5.2	37.0	4.1	87.0	3.8	4.86	99.7	4.1
4	0.2 per cent $(NH)_2SO_4$	0.6	6.7	8.9	5.5	44.7	4.1	45.6	4.0	115.5	3.4	6.26	159.6	3.7
5	0.2 per cent $(NH_4)_2SO_4$, 10 cc. H_2SO_4 per 100 gm. mixture	1.8	6.6	2.8	6.4	43.7	4.5	44.6	4.0	116.5	3.4	6.58	140.6	3.8

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.

† All composts contained 400 parts rock phosphate, 50 parts sulfur, 100 parts iron pyrites and 10 parts soil or soil and ammonium sulfate.

‡ Total P_2O_5 content was taken as 100 per cent.

phosphate, 50 parts of sulfur and 100 parts of iron pyrites, and 0.2 per cent ammonium sulfate. This latter salt was used to replace the nitrogen supplied by the 90 additional parts of soil used in the earlier experiments. The mixtures were incubated at 30°C. and at room temperature. Two tumblers in duplicate received 0.2 per cent of ammonium sulfate and to two of them was added also at the beginning of the incubation period 10 cc. of sulfuric acid per 100 grams of mixture. The pyrites had a lower pH value than the soil and rock phosphate, which resulted in making the mixtures slightly acid. Relative acidity and hydrogen-ion concentration were determined at intervals of two weeks and available phosphoric acid at the end of 10 weeks. The results are reported in table 3.

The influence of ammonium sulfate was noticeable from the beginning in the cultures kept at room temperature as well as in the cultures incubated at 30°C. The composts incubated at 30°C. which received 0.2 per cent of ammonium sulfate had accumulated an acidity at the end of 10 weeks equivalent to 132.2 cc. of 0.5 *N* NaOH as against an acidity equivalent to 92.1 cc. 0.5 *N* NaOH accumulated by the cultures with no ammonium sulfate.

The available phosphoric acid for these mixtures at the end of the same period was 9.54 per cent and 5.2 per cent respectively.

Here again the strength of the acid formed, as indicated by the lower pH values after 10 weeks of incubation, was greater than after 12 weeks, although the total acidity had increased considerably in the period between 10 and 12 weeks. It was at first thought that this was a mistake, but the figures given represent an average of 6 determinations. The measurement of hydrogen-ion concentration is a measuring of free acid at the time of the determination, and this changes continuously. Besides, the buffer action in the mixture may cause different readings at different times. Hydrogen-ion concentration measurements may indicate at which point of acidity accumulation phosphoric acid becomes available and in this way may afford a means to follow the progress of bacterial activities; but they have, naturally, no value as measurements of the quantities of acid and acid salts produced.

Series 4. Aeration

The aeration experiments reported in an earlier paper (10) were repeated with a compost consisting of soil, rock phosphate, sulfur, pyrites and ammonium sulfate as were used in series III. In addition, some of the mixtures which were continuously aerated received 10 cc. of sulfurous acid per 100 grams of the mixture at the beginning of the experiment, and others received similar quantities of sulfurous acid in the stream of moist air used for aeration. The apparatus used in the earlier work (10) was used to provide the mixtures with moist air. The mixtures which were placed in tumblers and to which 10 cc. sulfurous acid were added per 100 grams of mixture received these quantities at the beginning or by stirring into the mixtures during the first 8 weeks. All composts were placed at room temperature and kept in darkness.

The results obtained are reported in table 4. It is evident from this table that aeration of the mixtures by means of a stream of air had very little or no beneficial influence upon the availability of the phosphoric acid. Contrary to the experiments reported before (10) these aerated mixtures accumulated considerable acidity, provided ammonium sulfate was added. These particular mixtures had made available an average of 10.45 per cent phosphoric acid at the end of 10 weeks. This could not be attributed to the abundance of air acting on the pyrites for the cultures without ammonium sulfate received exactly the same amounts of air. The effect is ascribed to the pyrites acting as catalyser and thereby favoring the action of the sulfur-oxidizing organisms. As evidence for this assumption it may be stated that

TABLE 4

Influence of continuous aeration on the accumulation of acidity and available phosphoric acid

NUMBER	TREATMENT†	INITIAL REACTION		REACTION AFTER 2 WEEKS		REACTION AFTER 4 WEEKS		REACTION AFTER 6 WEEKS		REACTION AFTER 8 WEEKS		REACTION AFTER 10 WEEKS		REACTION AFTER 12 WEEKS		SOLUBLE P_2O_5
		*cc.**	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent*‡
1	Stream of moist air	0.6	6.7	4.6	5.9	12.4	5.6	16.8	5.1	22.4	5.0	36.2	4.8	59.4	4.2	0.62
2	0.2 per cent $(NH_4)_2SO_4$; stream of moist air	0.6	6.7	47.1	4.4	44.1	4.7	82.1	3.6	130.1	3.5	141.5	3.2	163.6	3.4	10.45
3	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added at beginning, stream of moist air	0.2	6.9	2.5	5.9	39.7	4.8	23.0	5.0	120.0	3.6	130.1	3.2	145.5	3.5	8.26
4	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid in container through which air was running	0.2	6.9	70.1	4.3	66.7	5.0	65.2	3.7	59.8	3.9	57.4	3.8	62.5	4.0	0.78
5	None	0.6	6.7	0.6	6.6	1.0	6.4	2.8	5.5	16.1	5.0	34.0	3.9	51.1	4.0	0.32
6	0.2 per cent $(NH_4)_2SO_4$	0.6	6.7	45.8	4.5	44.9	4.7	52.4	3.9	94.6	3.6	120.3	3.3	153.3	3.4	9.32
7	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added at beginning	0.2	6.9	4.0	5.7	42.8	4.8	57.1	3.9	49.7	3.8	53.2	3.9	130.4	3.5	7.81
8	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added by stirring	0.2	6.9	3.4	6.0	36.1	4.9	47.9	3.9	44.4	3.9	47.9	4.0	123.9	3.8	5.76

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.

† All composts contained 400 parts rock phosphate, 50 parts sulfur, 100 parts iron pyrites and 10 parts soil or soil and ammonium sulfate.

‡ Total P_2O_5 content was taken as 100 per cent.

qualitative analyses of the mixtures showed that great quantities of iron sulfates were present. This was undoubtedly due to the activities of the microörganisms, for but comparatively small amounts of iron sulfates were present in mixtures numbers 1 and 5 which received no ammonium sulfates as a source of nitrogen and which in consequence produced but little acidity.

The addition of sulfurous acid failed to stimulate these mixtures, both in the composts continuously aerated and in the mixtures kept in tumblers. Sulfurous acid stirred into the mixtures or added in the stream of moist air proved to be depressing rather than stimulating. The action of sulfurous acid therefore, seems in the first place of a sterilizing nature. Especially where small amounts of soil are used this action is more pronounced.

Series 5. Vegetation experiments

Since iron is one of the absolutely essential elements to normal growth and development of all agricultural plants, but since the quantities of iron in the soil are usually so large and supposed to be available in sufficient amounts to perform the necessary functions, it is not often applied as a fertilizer.

Strenuous efforts have been made by the manufacturers of certain products to introduce iron sulfate, which is a common by-product of a number of manufacturing processes, as a fertilizer. Pyrite cinders have been used in many places with success, especially in the Aisne region in France. Their value has been attributed both to the iron sulfate and to small amounts of nitrogen which these cinders contain.

Vivien (9) mixed 1 per cent of roasted pyrites with manure and found that considerable amounts of nitrates were formed, whereas in manure treated with acid phosphate, iron sulfate, calcium sulfate, and lime, only traces of nitrates were found.

Vermorel and Dantony (8) employed iron pyrites at rates of 100 and 200 kgm. per hectare as a top-dressing for wheat and beans. In one series they employed pure sand with additions of 50 kgm. sodium nitrate per hectare and in another series 100 kgm. dried blood per hectare. The pyrites was applied as a top dressing and mixed with the sand.

The pyrites increased the yields of wheat 40 per cent and for beans 50 per cent. These investigators conclude that the sulfur of the pyrites acted as a stimulant. They found however, that pyrites alone was more effective than sulfur alone on wheat and less effective on beans. Best results were obtained with a mixture of sulfur and pyrites.

Since these investigators had better results with a mixture of pyrites and sulfur the question can be raised whether this was due to the iron in a form more available to the plants. Van Alstine (6) concludes from his solution culture work that with a limited supply of iron in the form of ferric hydroxy-phosphate "a hydrogen-ion concentration of 4.5 is as low as can be expected to dissolve the amounts of iron necessary for proper growth of buckwheat, soy beans and wheat. With lower hydrogen-ion concentrations so little iron

is dissolved that these plants are unable to get the amounts they need and begin to show chlorosis as soon as the supply in the seed is used up." The form in which iron is supplied is very important as is shown by Jones and Shive (4) in their nutrient solution work. In the nutrient solution employed, iron in the form of ferric sulfate was very slowly and difficultly available to wheat plants even when supplied in relatively large quantities, but ferrous sulfate appeared to be readily available to these plants. It might be that the good results of Vermorel and Dantony with pyrites were caused by the iron changing from the sulfide into the sulfate form and thereby becoming more readily available, for they used paraffined pots with certain quantities of pure sand which was carefully freed from organic material. Through the washing of the sand they had naturally removed the iron present. That sufficient iron in the soil is not always available to plant growth is shown by the results obtained by Chanzit (2) with vines which showed chlorosis. He found that the presence of excessive amounts of $CaCO_3$ in the soil caused chlorosis to the vines, which he could overcome by applying 250–300 gm. of ferrous sulfate to each vine during the winter.

An experiment was carried on in the greenhouse in earthenware pots with washed quartz sand. Shive's nutrient solution (R_5C_2) was used as basis. The following treatments were used (the numbers refer to the pot cultures):

1. None.
2. Pyrites.
4. Nutrient solution (R_5C_2).
5. Nutrient solution and pyrites.
6. Nutrient solution, but phosphorus replaced by ground rock phosphate.
7. Nutrient solution, but phosphorus replaced by ground rock phosphate, and pyrites.
9. Nutrient solution, but phosphorus replaced by ground rock phosphate, inoculated sulfur, and pyrites.

The pyrites were added at a rate of 200 pounds per acre. The amount of rock phosphate employed was 2 tons per acre, calculated to be approximately the same as in the nutrient solution. Sulfur was added at a rate of 100 pounds per acre.

Soy beans were grown for 6 weeks and the yields obtained are recorded in table 5, together with other data obtained.

Although the yields of tops were but slightly higher for the cultures receiving pyrites in addition to the nutrient solution, they were markedly earlier in maturing. After 4 weeks all plants to which pyrites were added were blooming, while but one plant of the other cultures started to bloom at that time. This earlier maturing is also indicated by the number of pods produced after 6 weeks. The plants receiving inoculated sulfur and pyrites were dead after 18 days. The acidity produced was apparently too high for these plants.

Although no conclusions could be drawn from these experiments, since they were of a too limited scope, they seem to point to the possibility that at least a part of the increase in yields obtained by Vermorel and Dantony is caused by a change of the iron to more soluble forms, since no iron was applied at the beginning of the experiments, conducted by these investigators.

TABLE 5

Yields of soy bean tops grown in sand cultures for 6 weeks with Shive's nutrient solution as basis, and with additions of pyrites

POT NUMBER	TREATMENT	DRY WEIGHT OF TOPS	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION
	per acre	*gm.*	*cm.*		*pH*	*pH*
1	None	0.850*	8	Bloom	5.8	6.7
2	Pyrites, 200 lbs.	0.990	7.5	Bloom	5.4	6.5
3	R_8C_2	3.342	14	3	5.4	6.5
4	R_8C_2, pyrites, 200 lbs.	3.610	15	9	5.2	6.6
5	R_8C_2, rock phosphate, 2 tons; pyrites, 200 lbs.	2.022	10.5	2	4.9	6.4
6	R_8C_2, rock phosphate 2 tons; sulfur, 100 lbs.; pyrites, 200 lbs.	0.704	dead		5.0	3.3

* All measurements are an average of three cultures.

CONCLUSIONS

1. From these studies it seems evident that iron pyrites can be attacked by microörganisms and changed into the sulfate form. No attempt however, was made to study the intermediate steps in the changes occurring. If small quantities of sulfur are added these changes are much more rapid.

2. Pyrites composted together with sulfur and rock phosphate do not interfere with the gradual increase in acidity formation nor with the increase in availability of phosphoric acid.

3. The replacement of soil with ammonium sulfate in composts in which quantities of sulfur are replaced by iron sulfide produced a marked increase in available phosphoric acid. The effect, when sufficient nitrogen is present for the needs of the microörganisms, is ascribed partly to the pyrites acting as a catalyser and as such favoring the action of sulfur-oxidizing organisms, and partly to the changes from the sulfide into the sulfate form.

4. Aeration of sulfur-pyrites-rock phosphate compost mixtures by means of a continuous stream of air has little or no beneficial effect upon the production of acidity and consequent availability of soluble P_2O_5, unless ammonium sulfate is added.

5. The action of sulfurous acid in such mixtures seem to be mainly of a sterilizing nature.

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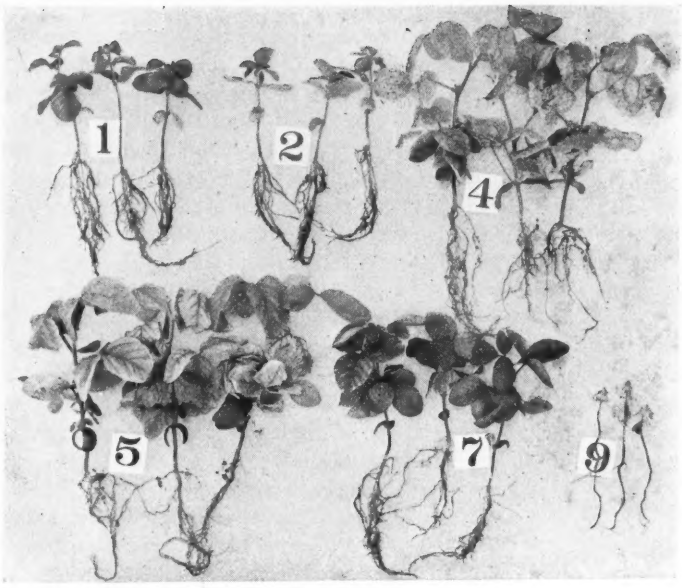
PLATE 1

PLANTS GROWN IN SAND CULTURES WITH SHIVE'S NUTRIENT SOLUTION AS BASIS AND WITH ADDITIONS OF PYRITES

Treatments were as follows: (1) none; (2) pyrites; (4) R_8C_2; (5) R_8C_2 and pyrites; (7) R_8C_2 and rock phosphate; (9) R_8C_2, rock phosphate-sulfur mixture and pyrites.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A MICROSCOPIC METHOD FOR DEMONSTRATING FUNGI AND ACTINOMYCETES IN SOIL[1]

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A few years ago a microscopic method for examining soil was proposed by the writer (2). Dried and stained microscopic mounts were used. Surprise was expressed at the time that practically no mold hyphae were observed in ordinary soils. It was remarked that there was no question but that fungi can live in the soil, and that the failure to find fungi in microscopic preparations did not disprove their activity in agricultural soils in general. In spite of such comments, however, the paper has been interpreted in some quarters as denying the activity of fungi in soils.

As the writer has always believed in their activity in soil under favorable conditions, it seemed well to continue the work by improving the methods of determining the presence of active fungi in soil, and to notice under what conditions they are most abundant. The criticism has been raised against the above mentioned microscopic technic that it might fail to show fungus filaments even if present—they might be torn off the slide in washing, they might be so affected by drying as to fail to stain, or the fungi might even occur in some vegetative form not easily recognized. That this criticism is not entirely justified may be shown by sterilizing a small quantity of soil in a test tube, inoculating it with mold spores of almost any species, and then after incubation examining under the microscope by the method in question. The presence of fungous hyphae is easy to demonstrate in any such inoculated soil, where they are known to be growing actively.

In spite of such findings, however, there seemed a possibility that there might be some truth in the claim that when but small quantities of filaments are present they may be washed off the slide and fail to show in the finished preparation. To meet this criticism, therefore, an improved technic has been developed, using wet mounts instead of dry. The technic is as follows:

Place a small crumb of soil (10 mgm. or less) on a microscopic slide. Mix it with two or three drops of water. Then dip a small glass rod into a methylen blue solution (either saturated aqueous or the Loeffler solution), and introduce the rod into the drop of soil infusion on the slide. Mix well together and cover with a cover slip, removing any sand grains that would prevent the cover slip from resting level. The strength of the stain on the slide should be such that the mount appears distinctly blue to the naked eye, but the field is

[1] This and the following paper appear together by mutual request of the authors.—ED.

only slightly tinted when viewed through the microscope. If too much stain has been added, it may be washed out without removing the cover glass by placing a drop of water on one side and touching the other side with a piece of filter paper. Examine with a dry lens and a highpower eye piece. The combination of lenses that has proved best for general purposes is a 16 mm. objective with a 15x compensating eye-piece.

By using this technic, fungus filaments, or at least fragments of them, have been observed in nearly all the soils that have been examined—a finding more nearly in accord with what was to be expected than was the observation made with the earlier technic. In the greater number of cases, however, the filaments were far from abundant. Sometimes in the whole preparation (comprising at least 5 mgm. of soil) only four or five fragments of mycelium were observed. The cases where they were found in sufficient abundance to suggest that they might have been playing a prominent part in the soil activities were those which would naturally be predicted; namely, where large amounts of undecomposed organic matter were present. By this same technic it was found that actinomyces filaments are abundant in some soils and entirely lacking in others, although plate counts always show large numbers of these organisms.

These observations emphasize even more than previous work the fact that plate counts of spore-forming organisms give no real idea as to their activity in any substance under investigation—be it soil or any other habitat of microorganisms. By using this microscopic technic it is possible, for example, to demonstrate an increase in the number of actinomycetes in soil in which young grass is growing. The plate count, to be sure, as pointed out some time ago (1), shows larger numbers of actinomycetes in sod than in cultivated soil; but this difference was never noticed until the sod was two or three years old. The microscope indicates that the increase in their activity takes place early in the life of the grass, but that their spores do not greatly increase in numbers for some time.

The promising feature in connection with the new technic is that it offers a rapid and apparently accurate method of determining the presence of vegetative of fungi and actinomycetes. The plate count gives misleading conclusions, as it is really a count of spores; the dried microscopic preparations show the filaments only when they are exceptionally abundant; the wet mount method is the only means yet at our disposal for getting direct evidence as to the extent of their vegetative activity. By using it, there should be no trouble in learning which soil conditions specially favor the development of these filamentous organisms.

SUMMARY

It is generally agreed that spores of fungi are universally present in soil and that they are capable of growing there under proper conditions. The presence of these spores in itself indicates activity at some recent date, as they are found too deep in the soil to be the result of air contamination without growth.

Naked-eye evidence shows that certain fungi, such as mushrooms, puffballs, etc., can thrive in soil, especially if it is well supplied with woody material or cellulose. This does not, however, necessarily indicate large activity of fungi in ordinary cultivated field soil to which little organic matter has been added, since the microscope shows mycelium to be present in but small quantities in such soil.

It must be acknowledged that fungi are always a potential factor, and possibly an important factor in soil fertility even though in ordinary cultivated soil their filaments may be few and their activities overshadowed by those of bacteria. In order to learn when and where they become active, instead of merely potential, factors in the soil activities, a simple method is needed for demonstrating the abundance of their vegetative forms, so as to correlate their abundance with the chemical transformations known to be occurring in soil. It is felt that the present technic supplies this much needed method.

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THE GROWTH OF FUNGI IN THE SOIL[1,2]

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A few years ago, the writer published a note (6) relative to the fact that fungi exist in the soil not only in the form of spores, but also as mycelium, which indicates that these organisms are active in the soil and take a part in the soil fertility processes. A method has been suggested for the demonstration of this fact, and, by the use of this method, which is quite simple, a number of fungi have been found to exist in the soil in the form of vegetative mycelium. The majority of the organisms isolated by this method were found to belong to the *Mucorales* and were included in the genera *Mucor*, *Rhizopus* and *Zygorhynchus*. Such large groups of soil organisms as the *Penicillia*, *Aspergilli* and *Cladosporia* were not obtained at all or only in very few instances by this method. This would point to one of two considerations: either the *Mucorales* develop more rapidly out of the soil and thus crowd out the other organisms or, that the *Mucorales* and a few other fungi, such as *Trichodermae* are present in the soil in the form of vegetative mycelium, while the *Penicillia*, *Aspergilli* and *Cladosporia* are present there only in the form of spores.

When the common plate method is used for the study of soil fungi, the opposite may be found to hold true: the *Mucorales* may not develop to such an extent as the *Penicillia*, *Aspergilli* and *Cladosporia*, while such fungi as the green *Trichodermae* are obtained by both methods. The following hypothesis then suggested itself: the *Mucorales* and *Trichodermae* are always present in the soil in the form of spores and vegetative mycelium, the various representatives of these groups preferring one or another soil type or different environmental conditions; the *Penicillia*, *Aspergilli* and *Cladosporia*, which are common air inhabitants and are found abundantly above the surface of the soil, in the dust, are always present, one group or another, in the soil in the form of spores, which may germinate and produce a vegetative mycelium, when soil conditions become favorable, as in the case of addition of organic matter, proper moisture content, etc.

Since the first note was published, several reports were made by the writer (7, 8, 9), in which a study was made of the fungous flora of the soil and it was

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[2] This and the preceding paper appear together by mutual request of the authors.—ED.

established without any doubt, that fungi (or molds) are permanent inhabitants of the soil and take part in important soil processes. Forms isolated in one locality may also be found in soils thousands of miles away, under entirely different environmental and soil conditions. However, the common plate method used with such convenience for the study of soil bacteria is entirely inadequate for the study of fungi, since not only the numbers but even the species of fungi developing on the common agar or gelatin plate do not give a true indication of the relative abundance and possible importance of fungi in the soil. This is due to the fact that the mycelium in the soil, however abundant, may be entirely absent, when the soil is diluted 10,000 or more times, for plating out of bacteria, while the fungi developing on the plate indicate only the spores of fungi present in the soil. These are so unevenly distributed that great variability is found not only throughout one soil sample but even on plates made from the same dilution, so that one plate may contain two or three colonies and a duplicate plate eight or ten fungus colonies.

In addition to the method suggested by the writer for the demonstration of fungus mycelium in the soil, a direct microscopic method has been suggested by Conn (2). The later investigator (1, 2), however, was unable to demonstrate any mold hyphae in the soil, which led him at first to conclude that molds are of relative insignificance in the soil. When one considers the fact that, for microscopic examinations, only a very small fragment of soil can be used and this is further diluted with water, one is not surprised that hyphae are not obtained by the microscopic method. Under these conditions any mycelium which would be present in a larger granule of soil, would be in most cases eliminated, since the mycelium is not so readily broken up into fragments as a bacterial chain. Then, mold hyphae assume in the soil a somewhat different appearance than in pure culture and might easily be mistaken, in a stained preparation, for organic matter.

The current idea about soil fungi was that these organisms are abundant only in soils of acid character and well supplied with organic matter. This is quite right, but it has also been pointed out in previous publications of the writer and others (3) that fungi are present in neutral and alkaline soils as well as in sandy soils containing very little organic matter. The following results will show that, 1, fungi are present in a mycelial stage in the soil and, 2, fungi are present abundantly not only in acid soils and those rich in organic matter, but even in nearly pure silicious sands not only in the form of spores but as abundant mycelium and also in neutral soils.

The author's method used for demonstrating fungi is, briefly, as follows: a clump of soil, the size of a large pea, taken out carefully from the soil sample with as little contamination from the air as possible, is placed in the center of a sterile plate, into which 10–12 cc. of a sterile nutrient agar, favorable for the development of fungi, has been placed; the soil is slightly pressed into the agar, so as to be surrounded by the nutrient medium. The plates are incubated at 25–30°C. At the end of 24–26 hours' incubation, the plates are

examined. Mold hyphae are then found to radiate out of the clump of soil into the surrounding medium. This is based upon the fact that the fungi present in the soil in the form of mycelium will grow at once into the medium, before the spores can germinate and develop hyphae. When a small piece of agar containing the growing mycelium, preferably a tip of a growing hyphae as far from the clump of soil as possible, is transferred upon a sterile slant of agar practically a pure culture of the particular fungus may be obtained.

One of the most common soil fungi found in the soil by the above method is a species of *Zygorhynchus*, closely related (5) to *Zygorhynchus vuilleminii* (Namys). A somewhat different species has been commonly found in northern soils obtained from Alberta (Canada) and Alaska. This fungus has been found by the direct method in practically all the soils examined, independent of the fact whether the soil is rich or poor in organic matter, acid, neutral or alkaline in reaction. Of special interest is the fact that this organism is found abundantly in subsoils, particularly sandy subsoils. Invariably a pure culture of this organism has been obtained by placing a clump of sandy subsoil, even as far as thirty inches deep, upon the plate.

Recently a sample of soil was received from Lakewood, N. J. It was obtained from an excavation and was stated to be present there in large quantities; its character was so unusual that an analysis was asked for. The soil belongs to the Lakewood series of sandy soils described in the soil Survey of the Freehold area (4), and is nearly a pure white sand, so typical of the New Jersey pine barrens. This particular sample, which was obtained in a sand excavation and was stated to represent a "considerable quantity" was found to be clumped together by a sort of a fine cottony mass penetrating throughout the soil. The soil had a reaction equivalent to pH 6.2 and contained, by the Kjeldahl method, 0.0123 per cent nitrogen.

This soil was examined for its content of microörganisms. The common plate method gave, in a 10,000 dilution, two or three bacterial colonies and two or three actinomycetes, per plate. A 1000 dilution gave fifteen to thirty bacterial colonies, six to ten actinomyces colonies, and one or two colonies of a green *Penicillium*. The direct method revealed the puzzle. A pure culture of the common soil *Zygorhynchus* was readily obtained and this was found to be the organism, whose mycelium penetrated the sand to such an extent that it held it in a compact mass. This organism which is found abundantly in the poor sandy subsoils of the Sassafrass series of New Jersey was found to grow to such an extent in this barren sand as to hold the sand together compactly. What rôle this organism plays in the soil is not yet known. It either decomposes the traces of organic matter present in the soil, making the nitrogen and minerals available for the scrub pines, or, as in the case of the subsoils, it merely thrives in this medium, practically free from other competing microorganisms, on the minerals washed down from the surface soil by the drainage waters. This fact, of course, would not point to any great importance of fungi in the soil, but tends to indicate that they may become readily active under favorable circumstances.

Another interesting instance of the activity of fungi in the soil is found in the following illustration. A farmer located a few miles from the Experiment Station observed the fact that, by mixing soil, undecomposed organic matter, such as clover, alfalfa, etc., and non-nitrogenous mineral fertilizer, then adding the proper amount of water, a good growth of molds takes place. When this mixture was turned over every two or three days, the molds decomposed the organic matter so rapidly that, in seven days, the whole mass was quite similar to leafmold, and compared favorable with prepared organic fertilizers for certain greenhouse plants. The farmer went so far as to apply for a patent on this process. When this mixture of soil, organic matter and mineral fertilizer was examined, it was found that a few species of *Mucor* (*M. plumbeus*, etc.), were chiefly responsible for the decomposition of the organic matter and for the artificial formation of the leafmold. The *Mucors* grew to a height of one to two inches above the surface of the mixture.

However, to be able to determine the actual number of fungi in the soil, as represented both by the numbers of spores and pieces of mycelium, a new procedure has been developed. This was a result of a series of studies on the variability of numbers of microörganisms in the soil as determined by the plate method (10). It was found that, when the numbers of fungi are determined on the plates prepared for the count of bacteria and actinomycetes, the variability is so great, due to the high dilution of the soil and, therefore, to the small numbers of fungi obtained, that the probable error obtained even from as many as 50 plates prepared from one soil is so large as to make the results worthless. Where low dilutions of the soil are employed, so many bacteria will develop on the plate as to actually crowd out a good many fungi and make the count entirely unreliable.

By the use of special acid media, on which no bacteria and actinomycetes will develop, and low dilutions (about 1000), the numbers of fungi can be determined quite accurately. Of the acid media two can be suggested:

1. Raisin agar, prepared by heating 60 gm. of raisins in 1000 cc. of tap water for 1 hour, then adding 25 gm. of agar, dissolving, adjusting the reaction to pH 4.0, filtering, tubing and sterilizing as usual.

2. Synthetic agar: 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 1000 cc. of water. Enough 1.0 *N* acid (sulfuric or phosphoric) is added to make the reaction equivalent to pH 3.6–3.8. This will require about 5–7 cc. 1.0*N* acid per liter of medium. Twenty-five gm. of agar are then added and dissolved by boiling. The medium is then filtered through cotton, tubed and sterilzied as usual. The reaction of the medium after sterilization should be pH 4.0.

By the use of the two acid media and a low dilution of soil (2–0.5 per cent of that used for counting of bacteria), plates can be obtained containing only fungus colonies. The number of fungus colonies per plate should be between 30 and 100. Incubate 48–72 hours at 25°C. Where the majority of the colonies are of the same species, there is ground for suspicion of air blown spores, rather than soil forms.

By the use of this method, not only were the numbers of fungi present in the soil found to be lower, but the results were more definite and less variable. Even more important, a definite correlation has been found between soil treatment, soil reaction and numbers of fungi in the soil, as indicated in the following table.

TABLE 1

Influence of soil treatment upon numbers of fungi as determined by the plate method

SOIL FERTILIZATION	REACTION	NUMBERS OF FUNGI PER GRAM OF SOIL
	pH	
Minerals only	5.6	37,300
Heavily manured	5.8	73,000
Sodium nitrate	5.8	46,000
Ammonium sulfate	4.0	110,000
Minerals and lime	6.6	26,200
Ammonium sulfate and lime	6.2	39,100

Manure and acid fertilizers (ammonium sulfate) stimulate an increase in the numbers of fungi. By making the reaction less acid, lime results in a great decrease in the numbers of fungi. Further information on this method and more extensive results will be published later.

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FIELD MOISTURE CAPACITY AND WILTING POINT OF SOILS

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The storage capacity of the soil for usable moisture under field conditions affects the amount of irrigation, necessary while the drouth, or wilting, point affects the time of irrigation and defines the lower limit of field capacity of the soil for usable moisture. The field capacity of the soil for usable moisture has come to be regarded, therefore, as one of its most important, if not the most important of physical properties. These matters have come to be given careful study in connection with Oregon Soil Investigations.

The wilting coefficient is the moisture content of the soil at which the plant wilts permanently or at which it cannot maintain its turgidity. This point has been regarded as varying but slightly with different plants, not usually more than 1½ per cent. It has commonly been regarded as varying widely with different soils. This wilting point represents the lower limit of available moisture. Briggs has shown (2) that it is approximately one and one-half times the hygroscopic coefficient. In coarse-textured soils the hygroscopic coefficient is very low and the wilting coefficient correspondingly low. In fined-texture soils these points are fairly high, the hygroscopic coefficient lying between 10 and 18 per cent, wilting occurring at 15 to 27 per cent. In some Oregon peat soils, the wilting coefficient has been found to be as high as 65 per cent. Oregon studies indicate that the wider the range in the different moisture points and the finer the texture, the greater is the difference between the wilting points of different crops grown on a given soil. With soils of coarse texture, where there is a narrow range of important moisture points, there is less difference in the wilting point of various crops. Oregon studies indicate that the difference is greater than was formerly supposed and reported (2).

WILTING POINT STUDIES UNDER FIELD CONDITIONS

The moisture history of dry-farmed and irrigated plats, including both meadow and cultivated plats, located on Willamette silty clay loam was obtained at the Oregon Station during 1910. From these data it appeared that the clover and other meadow crops did not require irrigation until the moisture content for the first two feet of soil dropped down to 14 or 15 per cent, dry weight. For potatoes the moisture content of the same strata did not

reach this low point during the entire growing season on dry plats, and the plats to be irrigated showed indications of drouth, when the moisture in the first two feet was still 20 or 21 per cent. An experiment was, therefore, planned determine the drouth point of potatoes and of clover, or the point at which it was best to apply irrigation water. A series of plats in duplicate was set aside, to be cropped to potatoes, one plat to be irrigated when the moisture content of the first two feet had dropped to twenty-three per cent point; the second when the moisture content reached the twenty-per cent point, and the third when the moisture had dropped to the seventeen-per cent point. After 1911

TABLE I.

Value of soil-moisture tests in determining the exact time to irrigate

CROP	TREATMENT	TOTAL YIELD PER ACRE	GAIN OVER DRY PLAT		GAIN PER ACRE-INCH OF WATER ADDED	WATER REQUIREMENTS
(1911)		*bu.*	*bu.*	*per cent*	*bu.*	*lbs.*
Potatoes (Recd. 3 by 3 inches)	Irrigated 23 per cent (or 9 inches)	292.5	157.4	117	17.5	1058
Potatoes (Recd. 2 by 3 inches)	Irrigated 20 per cent (or 6 inches)	308.5	173.4	128	28.9	799
Potatoes (Recd. 1 by 3 inches)	Irrigated 17 per cent	176.4	41.3	31	13.8	1326
(1913)						
Potatoes (Recd. 2 by 2 inches)	23 per cent	260.0	−40.5	−13.0	−10.1	973
Recd. 1 by 2 inches	20 per cent	342.0	41.5	18.0	10.4	655
Recd. none	17 per cent	300.5				629
(1911)						
Clover		tons	(green)		tons	
1 by 4 inches	20 per cent	17.05	6.60	63	1.85	338
1 by 4 inches	17 per cent	19.37	8.92	85	2.23	306
1 by 4 inches	14 per cent	19.62	9.17	88	2.29	303
(1913)						
2 by 5 inches	20 per cent	4.925		No dry plant,		514
1 by 5 inches	17 per cent	5.175		2nd crop year		539
1 by 5 inches	14 per cent	5.100				459

the points at which irrigations were applied were changed to 24, 21, and 18 per cent, respectively. At the same time clover plats were laid out to be irrigated which the moisture content dropped to the 20 per cent, 17 per cent, and 14 per cent points, respectively. An experiment was conducted for the years 1911, 1912, and 1913. Data were reported in Bulletin 122 of the Oregon Experiment Station. Since it is out of print, a summary of the more important data is presented in table 1.

In a summer of low precipitation, 1911, and in a summer of high precipitation, 1913, irrigation of potatoes when the moisture content reached 20 per cent seemed to give the greatest increases on the Willamette silty clay loam

soil, on the Corvallis experiment field, and this moisture content has come to be taken as an indicator of the exact time to irrigate this crop. During the same years it developed that clover was best irrigated when the moisture point dropped to 14 or 15 per cent for the first 2 feet; the moisture did more good when applied at these points, as the water requirement per pound of dry matter was generally lower under such conditions.

In order to check the drouth points of these crops and to eliminate any reinforcement of moisture from the subsoil secured by the deep roots of the clover plants, a series of Briggs type tanks were arranged in duplicate in 1918, and each of these crops grown in six tanks. Two tanks were irrigated when the crop was distinctly wilted, a second pair when the crop was but slightly wilted, and third pair when the crop was still in a fresh condition. Experiments were conducted in 1918 and the following year additional tanks were added for beets and alfalfa. After the crop growth was well under way, the tanks were allowed to dry down to the drouth point as indicated by the appearance of the crop at 3:00 p.m. Samples were then taken for soil-moisture determination from cores extending throughout the depth of the soil tank. The tanks were then given different amounts of irrigation to revive the plants and allowed to dry down until the average tank should again show slight wilting and the minimum tank distinct wilting, whereupon they were resampled. Representative tests are shown in table 2.

Some lack of uniformity in the data appears on account of interference of war conditions and the pressure of other work, but the average point at which the clover was found to wilt in these tanks was 16 per cent, for potatoes it was 17.5, for beets 20.3. This is the mean drought point by crops for this soil. The lower wilting point for all crops early in the season is due to low temperature (5) and relative humidity which was about 60 per cent, while later determinations were made with summer temperatures of 80° to 90°F. and a relative humidity of about 30 per cent. The water requirement was determined for potatoes and beets. The water requirement was generally lower for the crops in the average tanks. The data are sufficient to indicate strongly that the wilting point of the different crops varies more than has formerly been supposed. The indications are that it varies more widely in soils of heavy texture and wide range of important moisture points.

The wilting point as determined by laboratory methods for wheat seedlings in this Willamette silty clay loam has been found to be 15 per cent.

FIELD MOISTURE CAPACITY

The usable water capacity of nearly a score of important irrigated soils in different irrigated valleys of the state has been studied in connection with duty-of-water investigations (3) in order to secure definite information as to the best amount to apply at one time. Various factors affecting this field capacity have been measured. Cylinders 6 inches in diameter and 1 foot long.

or of approximately one-fifth cubic foot capacity were forced into the soil to a depth of 1 foot and the samples thus secured were saturated in a damp enclosure in the laboratory, drained to constant weight, and the total moisture content determined. This practice also afforded opportunity to determine the volume weight per cubic foot. Field samples taken for soil-moisture

TABLE 2

Wilting points of different crops

CROP	TANK NUMBER	COMPARATIVE MOISTURE AT SAMPLING	ACTUAL MOISTURE JULY 17	CONDITION OF PLANTS	ACTUAL MOISTURE AUG. 9	CONDITION OF PLANTS	ACTUAL MOISTURE SEPT. 10	CONDITION OF PLANTS	WATER REQUIREMENT
			per cent		*per cent*		*per cent*		*lbs.*
Clover	1	Min.	9.5	Firing	13.3	Dwarfed	17.8	Maturing	
	3	Min.	8.3	Firing	12.4	Dwarfed	16.3		
	3	Ave.	10.5	Wilting	17.3	Plump	16.5		
	4	Ave.	11.2	Wilting	16.2	Limp	18.5	Growing	
	5	Max.	12.4	Growth	21.4	Thrifty	16.3		
	6	Max.	13.0	Checked	20.4	Thrifty	16.3		
Average			10.8		16.7		17.5		
Potatoes	7	Min.	12.4	Firing	14.5	Wilted	15.9	Maturing	497
	8	Min.	14.9	Checked	13.5	Wilted	14.0		260
	9	Ave.	24.9	Not	19.0	Thrifty	17.2		278
	10	Ave.	20.0	Checked	15.3	Wilted	18.7		406
	11	Max.	20.5	Thrifty	16.9	Fair	17.7	Growing	456
	12	Max.	21.5	*Fallow*	18.0	*Fallow*	20.2		
Average			17.4		17.1		17.9		
Mangels	13	Min.	19.9	Limp	18.5	Wilted			580
	14	Min.	19.9	Limp	14.2	Some fired			501
	15	Ave.	20.0	Thrifty	19.7	Some wilted		Blighting	347
	16	Ave.	20.9	Thrifty	20.9	Plump			499
	17	Max.	26.5	Plump	21.7	Plump			486
	18	Max.	25.7	Plump	20.2	Plump			623
Average			20.4		20.3				
General Average			15.0		17.5		20.3		

determinations before and after irrigation have given indications of the maximum and minimum range of soil moisture under field conditions, and these, used in connection with the maximum field capacity tests and other data, aid in determining the usable moisture capacity in percentage and in inches on these important irrigated soils. The data so secured are presented in table 3.

In this table the humus content was determined by the ammonium method, and the moisture equivalent by the Briggs-Lane centrifuge or Briggs formula. In this table it appears that only the heaviest normal soils have as high a usable-water capacity as 2 inches per acre foot, these being silty clay loam, silt

TABLE 3

Relation of soil type and usable water capacity to irrigation requirement

LOCATION	SOIL TYPE	HUMUS	HYGROSCOPIC MOISTURE	MOISTURE EQUIVALENT	MOISTURE CONTENT		TOTAL WATER-HOLDING CAPACITY		WEIGHT OF OVEN-DRY SOIL PER CUBIC FOOT	ESTIMATED EXCESS POINT	ESTIMATED DROUTH POINT	ESTIMATED USABLE-WATER CAPACITY	
					Mean maximum	Mean maximum							
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*in.*	*lbs.*	*per cent*	*per cent*	*per cent*	*in.*
Paisley	Medium peat	60.00			140	35	147	7.6	28	110	40	70	3.8
Paisley	Peaty silt loam	10.00	3.60	9.756	50	18	64	6.5	52	45	20	30	2.5
Paisley	Silt loam	7.60			40	16	50	5.8	63	34	16	18	2.1
Corvallis	Silty clay loam	5.50	3.75	10.16	30	12	36	5.5	80	27	14	13	2.0
Talent	Silty clay loam		3.53	9.57	24				75	22	10	12	1.7
Burns	Silt loam		5.24	14.20	45	20	36	4.9	66	35	18	17	2.1
Haines	Loam	7.65	2.60	7.05	29	12	38	4.0	68	27	12	15	2.0
LaGrande	Loam	*3.20	3.50	9.49	30	10				27	12	12	1.7
Burns	Very fine sand loam	2.49	5.78	15.66	40	17	38	4.6	64	33	18	15	1.8
Haines	Fine sandy loam	5.89	2.30	6.23	30	10	33	4.9	68	26	12	14	1.8
Grants Pass	Fine sandy loam	2.50			18	8	23	4.3	85	18	9	9	1.5
Joseph	Fine sandy loam	8.85	2.20	5.96	40	10	47	5.7	64	30	15	15	1.9
Paisley	Sandy loam	2.18	3.76	10.19	28	12	30	4.7	84	22	12	10	1.6
Redmond	Medium sand		3.50	9.49	30	12	39	4.1	66	24	12	13	1.6
Lakeview	Medium sand				27	10	28	4.5	84	20	11	9	1.5
Paisley	Coarse sand	2.65	2.26	6.12	15	5	21	3.5	85	14	7	7	1.1
Hermiston	Coarse sand	trace			12	6	18	3.0	88	11	7	4	0.7

* Determined by Brigg's formula instead of centrifuge.

loam of high organic content. Peat, however, retains 3–4 inches of usable water per acre foot. The field capacity of usable water for silty loam soil is $1\frac{3}{4}$ inches; for sandy loam, $1\frac{3}{4}$–$1\frac{1}{4}$ for fine sand, about 1 inch; for medium sand, about $\frac{2}{3}$ inch; and for coarse sand as low as $\frac{1}{2}$ inch per acre foot.

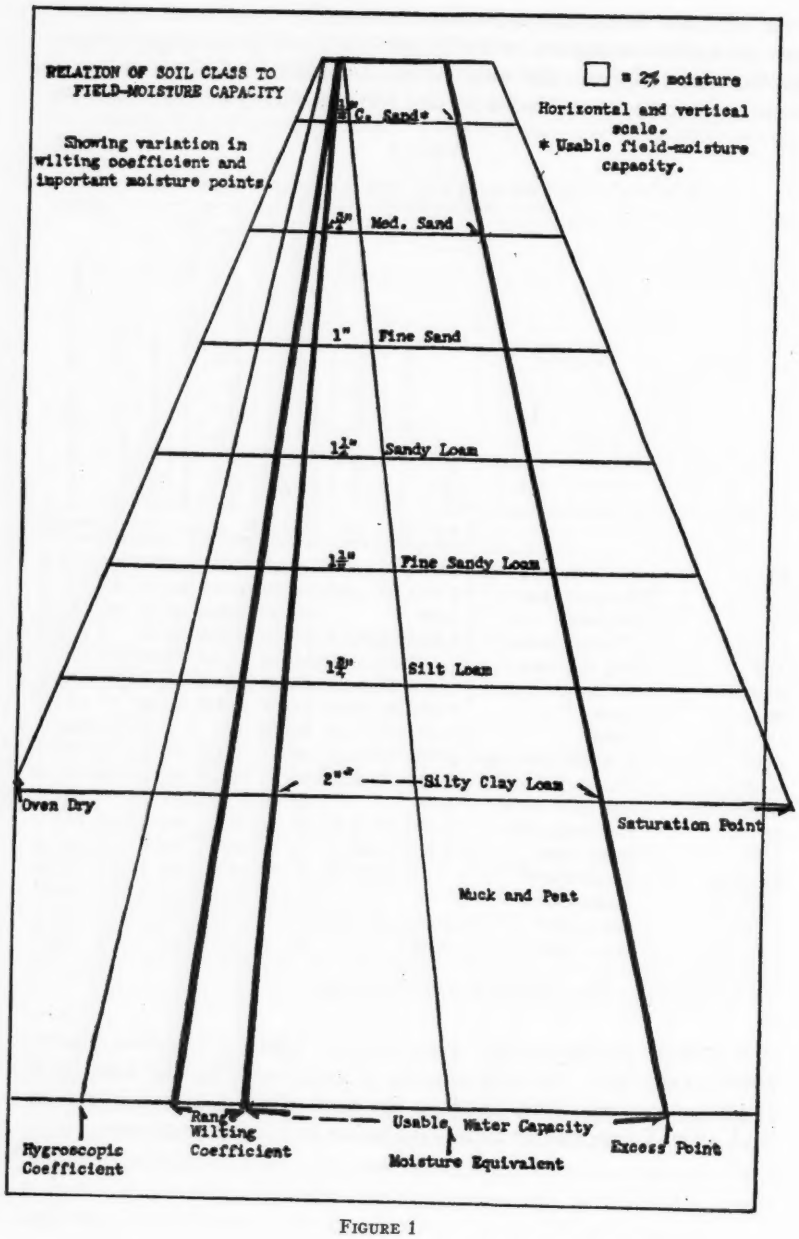
RELATION OF SOIL CLASS TO FIELD-MOISTURE CAPACITY
Showing variation in wilting coefficient and important moisture points.
□ = 2% moisture
Horizontal and vertical scale.
* Usable field-moisture capacity.
C. Sand*
Med. Sand
1" Fine Sand
Sandy Loam
Fine Sandy Loam
Silt Loam
Silty Clay Loam
Oven Dry
Saturation Point
Muck and Peat
Usable Water Capacity
Hygroscopic Coefficient
Wilting Coefficient
Moisture Equivalent
Excess Point

FIGURE 1

SUMMARY

The wilting point or drouth point is a valuable indicator in connection with the determination of the exact moisture content at which to irrigate (the purpose of which is to maintain a favorable soil moisture content).

The wilting point varies more between different crops than has commonly been supposed when judged by field and tank studies of crop appearance, soil moisture and yields of dry matter.

The wilting point appears to vary more for different crops on a soil which is rather heavy in texture than on a soil of narrow moisture limits.

The wilting point marks the lower limit of usable water. Both affect the time and amount of irrigation.

The usable-field-moisture capacity as judged by samples taken before and after irrigation, cylinder tests of field moisture capacity, physical composition, and determination of other moisture points shows that only the heavier classes of normal soils are capable of retaining as much as two inches of usable water in the surface acre foot. The coarsest soils used for irrigation retain only half an inch per foot of depth while peat retains three or four inches of usable water per acre foot.

The irrigation requirement is greater for soils of coarse texture and low humus content and is largely due to unavoidable waste in connection with light frequent irrigation.

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OCCURRENCE OF SULFIDES IN MINNESOTA PEAT SOILS[1]

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INTRODUCTION

The common occurrence of iron sulfide in peat has long been recognized. As early as 1810, Rennie in classifying peats (4, p. 160), described "pyritous, or vitriolic peat" (4, p. 640) and mentioned several even earlier writers, including Brougmart (4, p. 640) and Bomare (4, p. 642), who had not only recognized pyrite in peat soils but had also observed its behavior upon coming into contact with the air and the resulting toxicity to crop plants. He quotes Bomare (4, p. 643) to the effect that "the ashes furnish an excellent manure, though the moss itself when applied as a top dressing, is *utterly destructive to vegetation.*" Wollny in 1897 (7, p. 231) mentions the occurrence of iron sulfide in peat as marcasite as well as pyrite.

Pyrite and marcasite are formed where water carrying some iron compound comes into contact with a solution of calcium sulfate, under conditions which favor the reducing action of plant residues (7, p. 231, 3, p. 100). The sulfides themselves, being insoluble in water, are not toxic, but upon contact with oxygen and water vapor are converted into ferrous sulfate and sulfuric acid, both of which strongly affect plants and, when present in large quantities, destroy all vegetation.

As the sulfides are the source of the toxic substances, the amount of these, their distribution in the soil profile, and relation to soil layers rich in lime are of chief interest in the present discussion. The quantities of ferrous sulfate and sulfuric acid found at any time will depend upon the amount of sulfides present and their distribution as well as upon the aeration and the rainage conditions. Ditching and tiling, while facilitating the removal of these harmful substances, at the same time increase the aeration and hasten the oxidation of the remaining sulfides. So under the influence of drainage alone the toxic compounds will not disappear until the whole of the sulfide has been oxidized and there has been sufficient movement of water through the soil to leach out the oxidation products (1, p. 63).

Both ferrous sulfate and sulfuric acid are rendered harmless when any form of agricultural lime, either the carbonate, oxide, or hydroxide, is mixed with

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the toxic layer. It is not uncommon, however, to find these substances at the bottom of bogs in which the peat at the surface and for some distance downward is well supplied with lime, and in the mineral substratum of which, only a few inches below, these is an abundance of carbonate. In some cases, also, the same layer contains both carbonate and pyrites, but under this circumstance the toxic oxidation products will be neutralized almost as rapidly as formed.

In the reclamation of a bog the presence of a toxic layer becomes of importance since, if the peat is shallow, it may prevent the penetration of plant roots into the underlying mineral soil which otherwise might provide for any deficiencies of the peat in potash and phosphoric acid. Further, when material from such a toxic layer is thrown up in the course of ditching, and spread over adjacent land with the intention of improving it, it may prove injurious.

EXPERIMENTAL

In the autumn of 1918 several series of samples were collected from the Golden Valley Peat Experimental Fields in northern Minnesota. The chemical composition of the peat, the underlying muck and the mineral substratum is shown in table 1. In case of series A, B and C taken from the bank of a large drainage ditch adjacent to one of the experimental fields, it was found that the upper portion of the muck substratum and the section of peat immediately above it had a more strongly acid reaction, Truog method (6), than the layers above and below (table 2). From the surface down to this acid zone the peat was well supplied with lime, while the lowest part of the muck layer and the light-colored mineral substratum carried an abundance of calcium carbonate as shown by effervescence with dilute acid (table 2). Series D, taken from an excavation made in one of the experimental fields nearly a half mile back from the drainage ditch, did not show such marked differences in reaction.

The relative amounts of sulfide (table 2) were compared by using lead acetate paper. The procedure was briefly as follows:

> A 10 gm. sample was placed in an Erlenmeyer flask of appropriate size, 100 cc. of distilled water and a few cubic centimeters of concentrated sulfuric acid added, a strip of filter paper moistened with lead acetate solution placed across the mouth of the flask and the contents of the flask brought to a boil and the boiling continued just 2 minutes. The degree of blackening of the lead acetate paper, or *sulfide coloration*, indicated the relative amounts of sulfide present which were designated by the same terms as are used on Truog's standard color chart (6, p. 8) to express the degrees of acidity.

In the levels nearest to the dividing line between the peat and the muck, the samples from the ditch bank showed a greater degree of acidity than corresponding samples taken from the excavation. There was, however, no difference in sulfide coloration. Along the bank of the ditch, which had been dug seven years before, some of the sulfide had been oxidized with consequent formation of sulfuric acid. This accounts for the more acid reaction of the sections carrying the larger amounts of sufide. In general the most sulfide

TABLE 1

Chemical composition of Golden Valley peat

DEPTH OF SECTION	WEIGHT PER CUBIC FOOT	LOSS ON IGNITION	ASH	NITROGEN	LIME (CaO)	PHOSPHORIC ACID	REACTION BY TRUOG METHOD
in.	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
0–6	7.9	87.0	13.0	2.43	2.05	0.18	Very slight
7–12	9.9	80.0	20.0	2.46	3.29	0.20	
13–18	9.9	89.2	10.8	2.56	2.35	0.14	
19–24	12.6	88.2	11.8	2.57	2.39	0.15	Slight
25–30	13.9	87.4	12.6	2.96	2.21	0.15	Strong
31–33		32.8	67.2	1.23	2.12	0.14	Very strong
34–36		6.8	93.2	0.238	3.05		Neutral
37–39				0.163	8.99		
40–42				0.120	12.15		

TABLE 2

Reaction and relative amounts of sulfide in successive levels

Samples of 1918*

DEPTH	SAMPLES FROM DITCH BANK						SAMPLES FROM EXCAVATION	
	Series A		Series B		Series C		Series D	
	Acidity	Sulfide coloration	Acidity	Sulfide coloration	Acidity	Sulfide coloration	Acidity	Sulfide coloration
in.								
0–6	v. sl†	sl	v .sl	sl	v. sl	v. sl	v. sl	sl
7–12						v. sl	v. sl	sl
13–18						med	sl	med
19–24	sl	med	v. sl	med	sl	sl	v. sl	med
25–27	str	str	v. sl	med	sl	med	v. sl	sl
28–30					v. sl	v. sl	neut	sl (e)
31–33	v. str	med	sl	med	neut	sl (e)	neut	sl (e)
34–36	neut	sl (e)	sl	med	neut	v. sl (e)	neut	v. sl (e)
37–39			v. sl	sl	neut	v. sl (e)	neut	v. sl (e)
40–42			sl	sl	neut	v. sl (e)	neut	v. sl (e)
43–45	neut	....	sl	sl	....	....	neut	none
46–48	neut	....	neut	sl	....	....	....	....
49–51	....	....	neut	sl (e)	....	....	....	....
52–54	....	....	neut	v. sl (e)	....	....	....	....
55–57	....	....	neut	....	....	....	....	....

* Determinations were made 2 months after the samples were collected. The heavy cross lines indicate the approximate boundary between the peat layer and the underlying mineral soil.

† neut = neutral, v. sl = very slight, sl = slight, med = medium, str = strong, v. str = very strong, e = effervesces with dilute acid.

was found in the layer of peat immediately above the muck and not in the muck layer itself, just the opposite of Ramann's conclusion as to European peats. Ramann considered the sulfide more common in the muck layer underlying the peat than in the peat itself (3, p. 100).

In the samples dealt with, the sulfide occurred in such a finely divided state that it was not visible to the naked eye, and could be identified only by chemical tests. Typical samples showed the presence of iron while the two that were most strongly acid, those from the 28–30 and 31–33 inch sections of Series A, showed the presence of ferrous iron.

Two areas in the northwestern and one area in the northeastern part of the state were examined for sulfides and acidity during the season of 1919. Samples were collected only from the six-inch layer of peat immediately above the muck and from the upper portion of the muck layer. One of the northwestern areas extended from Golden Valley eastward some 6 miles and included the Golden Valley Peat Experimental Fields, of which a thorough examination was made, while the other, some 25 miles southeast of the first, embraced an area of shallow peat to the east of Thief River Falls. The one in the northeastern part of the state, was in the vicinity of Meadowlands, in St. Louis County, embracing an area 15 by 5 miles. In order to avoid any oxidation of the sulfide between the time of taking the samples and their laboratory examination, a field outfit was carried and the samples tested within a few hours after being taken. When shipped and later stored at the laboratory for some time before testing, samples had been found to show a more strongly acid reaction and less sulfide coloration.

Of the 20 series of samples taken from the Experimental Fields at Golden Valley in the second season, only one showed a strongly acid reaction in the peat layer, while all responded to the test for sulfides (table 3). Also the muck showed less acidity and less sulfide than the peat and in the case of three of the muck series, both acidity and sulfide were absent. This difference from the results of the first season is to be attributed to a flood (2, p. 51) following a torrential rain in the early part of July. Erosion of the walls of the large drainage ditch from which samples had been taken the year before widened it some 12 or 15 feet and exposed fresh sections on both sides. Samples taken from freshly exposed portions showed the same general condition as found farther back. There was no markedly acid peat or muck layer (table 3), and the sulfide coloration was about the same as the year before.

The samples taken at one-mile intervals east of Golden Valley and those taken east of Thief River Falls very closely resembled those taken on the Experimental Fields, in both reaction and sulfide content. Thus in general the results were very similar to those secured in 1918 with samples taken from the excavation on the experimental tract.

In only one place in the Meadowlands area was sulfide found. This was in a small "pocket" in which there was an unusual depth of muck—about two feet. In this instance the sulfide was more abundant in the uppermost por-

tion of the muck layer than in the peat. A summary of the samples tested and the number of these showing a sulfide coloration is given in table 4.

During the field work of the second season a set of 19 samples was collected for chemical analysis. These consisted of nine pairs of samples made up of the upper 6-inch layer of muck substratum and the 6-inch layer of peat immediately above this, taken at different places on the Experimental Fields at Golden

TABLE 3

Reaction and relative amounts of sulfide in peat soil from northwestern Minnesota

Samples of 1919

SERIES	PEAT		MUCK		SERIES	PEAT		MUCK	
	Acidity	Sulfide coloration	Acidity	Sulfide coloration		Acidity	Sulfide coloration	Acidity	Sulfide coloration
Golden Valley Peat Experiment Field					*Ditch Bank at Golden Valley*				
1	v. sl	sl	neut	v. sl	21	v. sl	v. sl		v. sl
2	v. sl	v. sl		sl	22	v. sl	v. sl	sl	v. sl
3	str	sl	sl	sl	23	v. sl	v. sl		none
4	sl	v. sl		none	24	v. sl	v. sl		v. sl
5	sl	sl		v. sl	25	v. sl	v. sl		sl
6	sl	v. sl		none					
7	v. sl	sl	v. sl	none	*East of Golden Valley*				
8	v. sl	sl		v. sl					
9	v. sl	sl		v. sl	26	v. sl	sl		sl
10	v sl	sl		v sl	27	v. sl	sl		v. sl
11	v. sl	sl		v. sl	28	neut	sl		v. sl
12	v. sl	sl	neut	v. sl	29	neut	sl		v. sl
13	neut	sl		sl	30	v. sl	sl		sl
14	neut	sl		sl	31	neut	v. sl		none
15	v. sl	sl		v. sl					
16	v. sl	sl		v. sl	*Near Thief River Falls*				
17	v. sl	sl		v. sl	32	sl	sl	neut	v. sl
18	neut	sl	neut	v. sl	33	sl	sl	neut	v. sl
19	neut	med	v. sl	sl	34	sl	sl	neut	v. sl
20	v. sl	sl		sl	35	neut	sl	neut	v. sl

TABLE 4

Number of samples showing the presence of sulfides

Samples of 1919

AREA	PEAT		MUCK	
	Number of samples collected	Number showing sulfide	Number of samples collected	Number showing sulfide
Golden Valley	50	50	51	46
Thief River Falls	4	4	4	4
Meadowlands	18	1	18	1

Valley, and of one sample of peat taken 4 miles southeast of Thief River Falls. To insure the exclusion of any air and the consequent oxidation of the sulfide present, the samples were packed firmly into 2-quart tin cans, tightly covered and sealed with paraffin. When the samples were opened in the laboratory the seals on all were found to be in good condition. The acidity and the sulfide coloration, as well as the amount of sulfide, expressed as H_2S, are reported in table 5. The iodine method (5, p. 398) used in determining the last is briefly as follows:

Twenty-five grams of moist peat or 50 gm. of muck is weighed into a 500-cc. Erlenmeyer flask, 100 cc. of distilled water and 10 cc. of stannous chloride added. The flask is then attached to a condenser in turn connected with another flask containing an ammoniacal solution of cadmium sulfate. Twenty cc. of hydrochloric acid is added to the contents of the flask through a separatory funnel and the whole brought to boiling and then gently boiled for 20 minutes. The contents of the flask containing cadmium sulfide are then nearly neutralized with hydrochloric acid and titrated with thirtieth normal iodine in the usual manner.

TABLE 5

Sulfide, reaction, and sulfide coloration of 19 samples of peat and muck

SAMPLE	PEAT			MUCK		
	Reaction	Sulfide coloration	Sulfide*	Reaction	Sulfide coloration	Sulfide
			per cent			*per cent*
4	sl	med	0.060	str	sl	0.006
1	str	med	0.058	neut	v. sl	0.002
5	v. sl	med	0.047	med	sl	0.014
3	sl	sl	0.037	neut	v. sl	0.005
9	sl	sl	0.036	str	v. sl	0.004
8	sl	sl	0.032	sl	v. sl	0.002
2	sl	sl	0.028	neut	v. sl	0.003
6	str	sl	0.025	neut	v. sl	0.003
7	v. sl	sl	0.016	v. sl	v. sl	0.002
10	sl	sl	0.016			

* Expressed as H_2S.

With both the peat and the muck there is a general agreement between the coloration of the lead acetate paper and the amount of sulfide found, i.e., the darker the sulfide coloration the larger is the amount of sulfide found. However, when a peat and a muck which show the same degree of sulfide coloration, are compared, no direct relation is shown between the degree of coloration and the amount of sulfide. The peat layer contains the larger amounts of the latter, varying between 0.016 and 0.060 per cent as compared with 0.002 to 0.014 per cent in the muck, and, for any given degree of sulfide coloration, shows a higher actual content of sulfide.

No concordance was found between the degree of acidity and either the sulfide coloration or the amount of sulfide the acidity being governed largely by conditions favoring oxidation and by the presence of carbonate.

Crops on the untreated plots on the Golden Valley field were comparative failures, with the exception of flax (2, p. 33), but when given a dressing of acid phosphate they yielded as well as on the surrounding mineral soil. On properly fertilized plots near the places where samples 1, 2, 5 and 6 were taken, the living plant roots were found to penetrate both the lowermost layer of peat and the muck, and even to extend down into the mineral subsoil. Thus either the amount of sulfide oxidized must be regarded as too small to harm the growing plants or the carbonate present neutralized most of the acid as rapidly as formed.

In a vegetation experiment with a pyrite-carrying muck from a shallow bog near Goodridge, 15 miles southeast of Golden Valley, clover plants on the untreated muck watered with distilled water made almost no growth, although at the end of a year were still alive. On the same muck treated with the ash obtained by burning a portion of the overlying peat and watered with tap water carrying large amounts of lime the growth of clover was excellent, the calcium carbonate in the ash and water being sufficient to render the two toxic compounds harmless. In the same Goodridge field complete reclamation was effected by the application of acid phosphate (1, p. 63).

In a similar vegetation experiment carried out in the plant house a year later with untreated muck from the Golden Valley Experimental Fields sweet clover did well when watered freely with the tap water.

On the surface of the peat in northwestern Minnesota, gypsum often occurs as a white incrustation sometimes so heavy that it crackles under the foot. It is also always to be found on the faces of ditches. A sample of the material intermixed with more or less peat from Golden Valley was subjected to repeated extractions with warm water until the leachings failed to give a test for sulfates, the extract evaporated on the water bath, and analyzed with the following results:

Fe_2O_3	0.0 per cent
Al_2O_3	0.0 per cent
CaO	28.5 per cent
MgO	4.1 per cent
So_3	55.5 per cent
Total Solids	0.1015 gram

SUMMARY

In samples of peat and muck from the Golden Valley Peat Experimental Fields in northwestern Minnesota in 1918, sulfides were generally found at all levels in the peat, in the muck substratum and in the upper portion of the mineral subsoil below. The greatest amount was found in the lowest portion of the peat layer and not in the muck.

The reaction of the peat and muck was found to be but little related to the relative amount of sulfides present, but to conditions permitting the oxidation of the sulfide to sulfuric acid and ferrous sulfate.

The layers in an exposed ditch face strongly impregnated with sulfide gave in 1918 a more acid reaction than did those at some distance from the ditch. But a year later, just after an unusual flood had widened the ditch and exposed fresh material, the peat of the ditch face was found to be similar in reaction to the corresponding layers farther back.

Expressing the sulfide content as hydrogen sulfide, nineteen samples of peat and muck showed a maximum of 0.060 and a minimum of 0.016 per cent for the lowermost layer of peat and 0.013 and 0.002 per cent for the muck substratum immediately below.

Sulfides appear to be much more commonly associated with peat in northwestern than in northeastern Minnesota, where in an area of approximately 75 square miles, sulfides were found at only one place.

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